THE ECOLOGICAL IMPACTS OF SIGNAL CRAYFISH IN UPLAND STREAM ECOSYSTEMS

Shams M. Galib

ABSTRACT

Non-native species are an important driver of global biodiversity loss. Worldwide, crayfishes are one of the prominent groups of non-native species. In this study, the American signal crayfish *Pacifastacus leniusculus*, the most widespread non-native species in Europe, was used as a model invasive crayfish species to determine the impacts and factors driving the dispersal of non-native species in upland stream ecosystems of northeast England.

Strong impacts of signal crayfish on stream biota over short (~7 weeks), medium (7 years) and long (28 years) timescales was evident through a combination of controlled mesocosm study, field surveys of a large number of streams and historical data. Density-dependent impacts of crayfish on multiple components of ecosystems including algal growth, leaf litter decomposition, macroinvertebrates and benthic indigenous fish were revealed. Stable isotope analyses showed a significant change in the trophic position of benthic fish in relation to crayfish density but it remained unchanged for crayfish. Decreased abundance of benthic fishes and young-of-year salmonids were recorded over time in crayfish-invaded streams whereas an opposite trend was recorded in uninvaded streams. Benthic fish disappeared in two invaded streams. Three uninvaded streams were invaded between 2011 and 2018. Dramatic declines in macroinvertebrate abundance and taxonomic richness were recorded in invaded streams and stream reaches compared to uninvaded controls.

This thesis also identified the factors driving the dispersal of invading crayfish in upland streams through the analysis of crayfish personality, propagule pressure and habitat suitability. Study of three population conditions (fully-established, newly-established and invasion front) revealed that crayfish dispersal in invaded habitats is context dependent. Personality traits played an important role in dispersal, especially at the invasion front but other factors including local population density and availability of refuges also play a key role. Apart from conventional personality traits (e.g. activity, distance moved and exploration), climbing ability, a trait that has received less attention in behavioural studies, was found to influence crayfish dispersal at newly-established and invasion front sites.

Currently, no single method is effective in controlling the spread of non-native crayfish to new sites, and at locations where invasive crayfish already exist. Therefore, improvement of existing legislative measures and raising awareness through education are very much needed to reduce intentional and unintentional introductions. In invaded habitats, if early detection is possible, damage can, potentially, be minimised through existing control methods. In-stream barriers may offer promise in controlling crayfish invasion in streams but this requires further research to validate and optimise designs. Findings of this thesis have contributed to our understanding of biological invasion, especially in upland stream ecosystems and underline the importance of managing crayfish invasion.

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List of abbreviations

AIC	: Akaike Information Criterion
AIS	: Aquatic Invasive Species
BA	: Before–After
BACI	: Before-After-Control-Impact
BMWP	: Biological Monitoring Working Party
CBD	: Convention on Biological Diversity
CI	: Control–Impact
CL	: Carapace length
DEFRA	: Department for Environment Food and Rural Affairs (UK)
DTDF	: Diet-Tissue Discrimination Factor
EA	: Environment Agency
EASIN	: European Alien Species Information Network
FE	: Fully-established (site of Westholme Beck)
GISP	: The Global Invasive Species Programme
GLM	: Generalised Linear Model
IAS	: Invasive Alien Species
ICC	: Intraclass Correlation Coefficient
IF	: Invasion front (site of Thorsgill Beck)
IUCN	: The International Union for Conservation of Nature
LMM	: Linear Mixed-Effects Modelling
NE	: Newly-established (site of Thorsgill Beck)
NMDS	: Non-Metric Multidimensional Scaling
PCA	: Principal Component Analysis
PERMANOVA	: Permutational Multivariate Analysis of Variance
PVC	: Polyvinyl chloride
SIMM	: Stable Isotope Mixing Model
SIMPER	: Similarity Percentage Analysis
SMRT	: Sterile Male Release Technique
WCC	: White-clawed crayfish
YoY	: Young-of-year

Declaration

The material contained within this thesis has not previously been submitted elsewhere for any other degree or qualification. The research reported here has been conducted by the author unless stated otherwise.

(Shams M. Galib)

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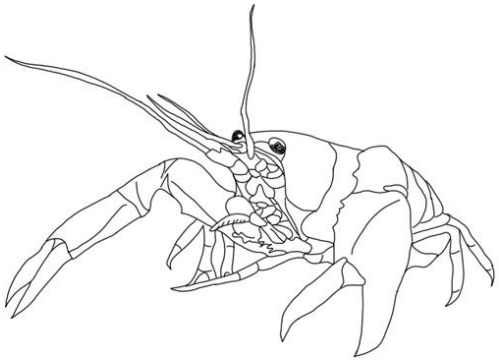


Chapter One

GENERAL INTRODUCTION

1.1 Global biota: an overview

The existence of life and its diversity is one of the extraordinary features of Earth which supports approximately 9 million types of biota in the form of plants, animals, protists and fungi (Cardinale *et al.*, 2012). Diverse communities tend to be more productive and resilient to change in natural functioning (Cardinale *et al.*, 2012) but ecosystems around the globe are rapidly losing biodiversity due to both anthropogenic and natural causes (SCBD, 2006; Naeem *et al.*, 2012). Current rates of extinction of species are about one thousand times the likely background rate of extinction (Pimm *et al.*, 2014). A wide range of specific causes for this loss have been identified including increasing use of natural resources by humans, modification or loss of habitats, climate changes, alien species, and spread of pathogens, domestic plants and animals (Naeem *et al.*, 2012).

Freshwater ecosystems occupy less than 1% of the world's surface but support ~10% of all known species including 33% of the vertebrate species (Strayer and Dudgeon, 2010). Among the different ecosystems, especially compared to terrestrial and marine ecosystems, loss of biodiversity is higher in freshwater ecosystems, making freshwater conservation a priority (Richman *et al.*, 2015). Freshwater resources provide a range of important services including domestic and commercial water supply, fisheries, carbon sequestration and energy but the ever expanding global human population is severely impacting these resources leading to a crisis in freshwater biodiversity (Vörösmarty *et al.*, 2010). As a consequence fishes and amphibians have become more vulnerable to extinction risks when compared to terrestrial vertebrates like mammals, reptiles and birds (Cumberlidge *et al.*, 2009; Darwall *et al.*, 2011; Holland *et al.*, 2012).

Serious threats to freshwater ecosystem stability and biodiversity have already been recognised which result in loss of habitats and biodiversity throughout the world (Williams *et al.*, 1989; Cowx, 2002; Suski and Cooke, 2007) (Table 1.1). All these threats can be grouped into five major categories, overexploitation, water pollution, flow modification, habitat degradation and biological invasion (Dudgeon *et al.*, 2006) (Figure

1.1) and there is a need for a better understanding of the factors causing these declines (Shea and Chesson, 2002; Suski and Cooke, 2007).

TABLE 1.1 Important threats to freshwater habitats and biodiversity and their impacts across the globe, modified from Suski and Cooke (2007).

Threats	Severity	Major impacts
Species introduction	High	Compete with native species directly or indirectly. Some predate on native species; alter nutrient composition, nutrient cycling and habitat structure
Physical barriers (e.g. dam, weir etc.)	High	Separate population, prevent migration and dispersal. Also disturbs overall hydrological patterns
Global warming / climate change	High	Can affect the physiology of aquatic life due to increased water temperature
Commercial harvest	High	Excessive harvest may negatively affect the populations
Physical habitat damages (e.g. channel straightening, dredging etc.)	High	Alter spawning and feeding grounds, and water temperature
Artisanal fishing	Moderate	Indiscriminate exploitation can badly impact aquatic populations
Flow regulation	High	Usually negatively affects the habitat and its populations
Pollution / Eutrophication	High	Alter nutrient levels and disrupt food web
Acidification	High	Records of damaging habitats are available
Ultraviolet (UV) radiation	Moderate	Can affect littoral habitats and organisms

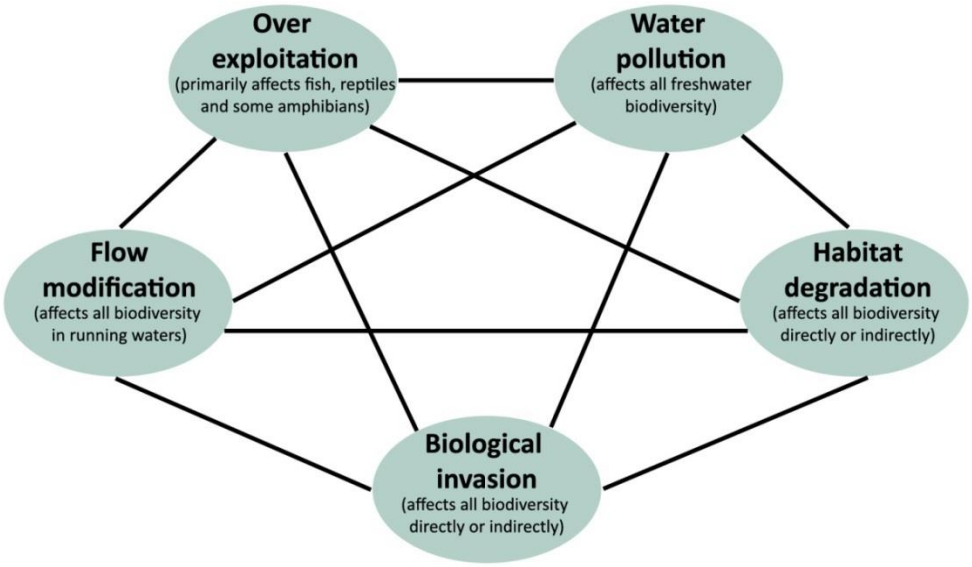


FIGURE 1.1 Major threat categories and their interactive impacts on freshwater biodiversity (modified from Dudgeon *et al.*, 2006).

1.2 Invasive species: one of the reasons for ecosystem change

Among the various reasons for declining biodiversity worldwide, as mentioned earlier, biological invasion of non-native species is playing a key role (Lodge, 1993; Naeem *et al.*, 2012; Caffrey *et al.*, 2014; Veale *et al.*, 2015). Invasive species are a subgroup of non-native species with unusually strong colonisation tendencies and can be responsible for serious environmental, economic and human health impacts (Keller *et al.*, 2011). Invasive species are considered, after land-use change, to be one of the most critical key drivers of ecosystem change and in the modification of biological communities (Mack *et al.*, 2000; Sala *et al.*, 2000). Colonisation by invasive species is one of four primary threats (cf. five; IPBES, 2018) to biodiversity at a global scale (Mora and Sale, 2011) and the first or second-ranked threat to freshwater biodiversity and ecological function in most parts of the world (Lodge *et al.*, 2000; Sala *et al.*, 2000). Aquatic Invasive Species (AIS) impacts are especially widespread. Water bodies affected by human activity and modification are more susceptible to invasion by introduced species and presence of exotic (non-native) species can be

used as an indicator of degraded conditions (Kennard *et al.*, 2005).

Biological invasion can alter food web structure by decreasing species richness and the number of links per species, posing a threat to ecosystem integrity and functioning (Gherardi *et al.*, 2009; Galiana *et al.*, 2014). According to Cardinale *et al.* (2012) "ecosystem functions are ecological processes that control the fluxes of energy, nutrients and organic matter through an environment." The fundamental nature of these processes and the rapid spread of non-native species has led scientists to increase the intensity of research on different aspects of biological invasion and invasive species.

It has been estimated that 480000 non-native species have been introduced into various habitats around the globe (Pimentel *et al.*, 2001). About 2000 to 50000 species have been introduced into six countries (UK, US, Australia, South Africa, India and Brazil) (Pimentel *et al.*, 2001). Also there are undetected introductions in many countries (Lodge, 1993). Important pathways of non-natives introduction are summarised in Table 1.2. Species introduction into inland waters is positively associated with the degree to which people utilise these habitats for recreation, food sources and commerce (Rahel, 2000). However, not all introduced species become invasive. According to Williamson (1996) the fates of introduced organisms can be of the following three types: (i) introduced but fail to establish self-reproducing populations, (ii) introduced and established, but at low densities or with little impact on indigenous community and (iii) introduced, successfully established and exert a large impact on native species or ecosystems. It has been estimated that around 10% of introduced species become invaders or establish themselves successfully (Williamson and Brown, 1986; Williamson and Fitter, 1996). Not all introduced organisms have equal potential to become invasive and this process is influenced by genetic, demographic and ecological factors (Allendorf and Lundquist, 2003). Nevertheless, it has been demonstrated that an invader species is more likely to succeed in a species-poor community than in a species-rich community (Lodge, 1993). From the very beginning of invasion biology, the study of the factors associated with the success or failure of invasions has been a central goal (Elton, 1958). Study of different aspects of invasive

species is essential in this regard.

TABLE 1.2 Important pathways of introduction for common non-native animal and plants, adapted from Keller *et al.* (2011).

Group	Pathways
Terrestrial vertebrates	
Mammals	Intentional introduction (e.g. for hunting, as pets, for zoo etc.) followed by either intentional release or accidental escape
Birds	Intentional introduction (e.g. for hunting, as pets, for zoo / bird parks etc.) followed by either intentional release or accidental escape
Reptiles / amphibians	Intentional introduction (e.g. as pets, for fauna improvement, food source, biological control agents etc.) followed by either intentional release or accidental escape
Terrestrial invertebrates	
Insects	Unintentional (as contaminants or stowaways) or intentional (as biological control agents) introduction
Others	Unintentional introduction (as contaminants or stowaways)
Terrestrial plants	
Vascular plants, mosses and lichens	Intentional (for ornamental purposes or horticulture) or unintentional (as contaminant of agricultural or ornamental plants) introduction.
Aquatic biota	
Fishes	Intentional (for aquaculture, ornamental or recreational fisheries, as biological control agents, illegal stocking) or unintentional (escape of ornamental fishes, fishing bait releases etc.) introduction
Crustaceans	Intentional (for aquaculture, ornamental fisheries) or unintentional (with ship ballast water, canals) introduction
Molluscs	Unintentional introduction (with shipping, waterways, from garden ponds or aquarium trade)
Plants	Intentional introduction (ornamental trade), often spread is facilitated by boats and waterbirds

The impact of non-native aquatic species can be severe, altering ecosystems, leading to the loss of native species, and having major economic outcomes such as harming fisheries (Pimentel *et al.*, 2001; Keller *et al.*, 2011; Sandodden *et al.*, 2018). Non-native invasives are a concern for conservation too; endemic species are now facing more alien species,

including in aquatic habitats (Lasram and Mouillot, 2009). Non-native species were considered in 2002 to be at least partially responsible for an estimated one-third to one-half of the world's crayfish (subphylum: Crustacea, order Decapoda, infraorder Astacidea) species being at risk of serious population decline or extinction (Taylor, 2002). Forecasting the consequences of species invasions is important in directing management and control efforts. However, it is often the case that the effects of invasive species are overlooked in widely established biodiversity management plans such as Protected Areas (PA) but issues like harvesting of species and habitat loss are being focused upon (McClanahan *et al.*, 2002). This type of ignorance needs to be addressed because invasive species can exert devastating effects on populations as much as do harvesting and habitat loss (Manchester and Bullock, 2000; McClanahan *et al.*, 2002; Mora and Sale, 2011; Wilkinson, 2012; Simberloff *et al.*, 2013). Globally, it has been speculated that dealing with invasive species might be the biggest challenge for conservation biologists in the next few decades (Allendorf and Lundquist, 2003). So the study of invasive species has become a burning issue in ecological research.

1.3 Invasive species in Europe and the UK

As a result of increasing international trade and travel, a remarkable increase in the movement of non-native species over the world took place in the last century (Hulme *et al.*, 2009). This trend is also true for Europe and is depicted in Figure 1.2. Illegal introduction of several fish and shellfish species has also taken place to increase the number of species of interest, mostly for recreational purposes i.e. to be used for angling or as a bait or as 'forage' (Gherardi *et al.*, 2009).

There are 12000 non-native species in Europe (c.f. >14000 species; Katsanevakis, 2015; EASIN, 2020) of which 11% are considered invasive and responsible for environmental, economic and social damage (Caffrey *et al.*, 2014). A study revealed that 63% of the introduced aquatic species were established successfully in six European countries (United Kingdom, France, Spain, Sweden, Germany and Italy; García-Berthou *et al.*, 2005). These species cause an annual economic cost of €12 billion for the EU

(Brink and Shine, 2008), £1.7 billion for Great Britain (Williams *et al.*, 2010) and €261 million for Ireland (Kelly *et al.*, 2013). However, it is assumed that severity of biological invasions in Europe will increase in future (Caffrey *et al.*, 2014).

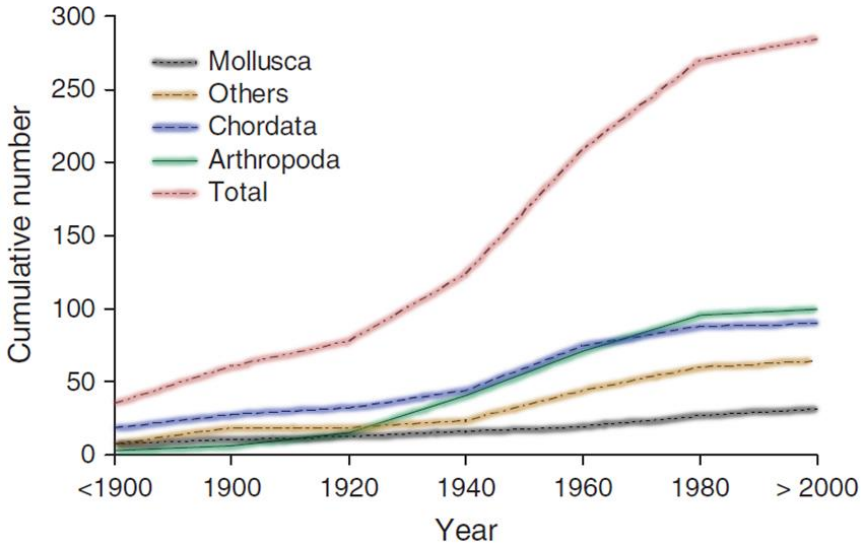


FIGURE 1.2 Non-native species in European inland waters. Dates refer to the exact or approximate year of introduction into the wild or, when this datum is absent, to the year of the first record in the published literature. The date is missing for 22 species (source: Gherardi *et al.*, 2009).

In Europe, effective control of invasive alien species has been hampered for three main reasons: (1) inadequate monitoring for alien species at frequent enough intervals in regions of concern; (2) lack of an effective means to report, verify the identifications, and warn of new sightings; and (3) lack of risk assessments that predict the likelihood of a particular species becoming invasive (Hulme *et al.*, 2009). Most of the exotic fish and shellfish species were introduced intentionally in Europe (Gherardi *et al.*, 2009) as in many other parts of the world (e.g. Rahel, 2000; Rahman, 2005; Ellender and Weyl, 2014) with a view to improving aquaculture, stock enhancement, ornamental purpose, biocontrol etc. (Gherardi *et al.*, 2009; Nunes *et al.*, 2015). Unfortunately, most of the planned introductions of fishes and invertebrates in inland waters of Europe have been carried out with no scientific basis (Gherardi *et al.*, 2009).

In the UK alone, there are estimated to be almost 2000 non-native

species across all ecosystems, costing £1.7 billion in management costs and damage per annum (Roy *et al.*, 2012). In Britain, the majority of the established exotics are higher plants (1376 species) followed by insects (344 species), non-insect invertebrates (158 species), vertebrates (50 species), algae (24 species) and lower plants (6 species) (Roy *et al.*, 2014). This makes it one of the countries with the highest numbers of established alien species in Europe, along with Spain, Italy and France (Essl *et al.*, 2013). Among the top-ranked invasive species in freshwater systems across Europe, including in the UK, are non-native crayfishes (Oficialdegui *et al.*, 2020). A considerable amount of money and effort have already been used for the control of invasive crayfish species (Rogers and Holdich, 1998; Holdich, 1999; Lodge *et al.*, 2000; Peay, 2001). A total of 12 species of crayfish are considered invasive in different parts of Europe and this number is eight in the UK (Holdich *et al.*, 2014; Kouba *et al.*, 2014). However, in the UK, signal crayfish (*Pacifastacus leniusculus*, Dana) is by far the most widespread and its management costs £2.69 million every year (Williams *et al.*, 2010). So, all these issues urge more research on crayfishes for the development of effective management strategies.

1.4 General overview of crayfish biology with reference to crayfish species in the UK

Crayfish (infraorder Astacidea) belong to the Decapoda, which is the largest crustacean order (Holdich, 2002). There are two superfamilies, the Astacoidea and the Parastacoidea (Hobbs, 1988). There are over 640 species of freshwater crayfishes across the world (Crandall and Buhay, 2008), including 38 in the Palaearctic region (Crandall and Buhay, 2008), although the signal crayfish is one of the most widespread (Kouba *et al.*, 2014). Crayfish are among the largest mobile freshwater invertebrates and tend to be nocturnal, using daytime refuges to reduce susceptibility to diurnal predators (Bubb *et al.*, 2002; Holdich, 2002). Invasive crayfishes are also large, mobile, omnivorous and carriers of pathogens (Unestam, 1972; Vogt, 1999; Cerenius and Edsman, 2002; Holdich, 2002) and are thus appropriate model species to examine key mechanisms of impact upon

recipient communities.

Being very adaptive, both physiologically (McMahon, 2002) and behaviourally (Gherardi, 2002), signal crayfish occur across a diverse range of habitats from lentic to lotic waters, in subterranean and semi-terrestrial environments and also into coastal waters (Nyström, 2002). Non-native crayfish can be so physiologically tolerant that they can behave normally even after consuming toxic substances including hepatotoxic cyanobacteria which they accumulate and store and may transfer into food chains, as observed in invasive signal crayfish (Lirås *et al.*, 1998). Several species of crayfish have been recognised as invasive in different countries, e.g. *Procambarus clarkii* in Spain (Gherardi and Barbaresi, 2000), *P. leniusculus* in the UK (Bubb *et al.*, 2004) and *Faxonius rusticus* in the US (Larson *et al.*, 2019). Yet, crayfishes are one of the most globally threatened taxa too (Westhoff and Rosenberger, 2016). The literature suggests that there is one native and eight non-native crayfish species in the UK (Table 1.3), although not all of these may be extant in the wild currently. However, no crayfish species is native to Scotland (Maitland, 1996).

TABLE 1.3 Crayfish species in the UK (sources: Holdich *et al.*, 2004 and 2014).

Common name	Latin name	Status in the UK
Native or white-clawed crayfish	*Austropotamobius pallipes*	Indigenous
Signal crayfish	*Pacifastacus leniusculus*	Introduced
Turkish/narrow-clawed crayfish	*Astacus leptodactylus*	Introduced
North American red swamp crayfish	*Procambarus clarkii*	Introduced
European noble crayfish	*Astacus astacus*	Introduced
North American spiny-cheek crayfish	*Orconectes limosus*	Introduced
Virile crayfish	*Orconectes virilis*	Introduced
White river crayfish	*Procambarus acutus*	Introduced
Australian redclaw	*Cherax quadricarinatus*	Introduced

In the UK the white-clawed crayfish (WCC) is an endangered crayfish species with a restricted range in Europe and whose current population status is 'decreasing' (Füreder *et al.*, 2010). It is the only crayfish native to Great Britain and Ireland (Holdich and Rogers, 1997). In the UK, a gradual decline in the WCC crayfish population took place after the mid-1970s due to pollution and habitat degradation (Almeida *et al.*, 2014). More recently crayfish plague (infection with the pathogenic agent *Aphanomyces astaci* of the Family Leptolegniaceae can cause mass mortality of native crayfish) and competition with non-native crayfishes, especially signal crayfish, adversely affected the native WCC population. This species is protected under the Wildlife and Countryside Act 1981 (Holdich and Reeve, 1991).

1.5 Impacts of non-native crayfish on the environment and biota

Due to their large adult size, behaviours and wide dietary spectrum, crayfish may impact, directly or indirectly, multiple components in streams and rivers and can have wide-ranging negative effects on habitats, native flora and fauna (Rabeni *et al.*, 1995; Holdich, 1999). Non-native crayfishes are now considered a major threat to freshwater biodiversity and ecosystem functioning (Twardochleb *et al.*, 2013). The impacts of non-native crayfish species on different components of ecosystems are summarised below.

1.5.1 Impacts on physical characteristics of habitat

Non-native crayfish including signal crayfish can effectively modify physical characteristics of the habitat. They can burrow into the bank and river bed (Guan, 1994; Holdich, Rogers, *et al.*, 1999; Lewis, 2002) and can be responsible for rapid water leakage and loss of moisture in the substrate (Souty-Grosset *et al.*, 2014). Signal crayfish are also capable of moving and mobilising sediment, particularly small particles (Harvey *et al.*, 2011, 2014; Rice *et al.*, 2012; Albertson and Daniels, 2016; Mathers *et al.*, 2019). Invasive red swamp crayfish *P. clarkii* are also reported to cause damage to levees, dams and water control structures in water bodies within

agricultural areas by its burrowing activity (Horwitz, 1990). Crayfish burrowing activity can lead to agriculture production loss, for example rice (Arce and Diéguez-Uribeondo, 2015), channel bank erosion and increased turbidity of water (Anastácio and Marques, 1997; Rodríguez *et al.*, 2003). Interestingly, invasive species can modify the environment in such a way that it may become unfavourable to them, as recorded for invasive rusty crayfish (*F. rusticus*) in lakes of the US (Larson *et al.*, 2019). As activities of signal crayfish increase turbidity through mobilisation and suspension of greater amount of fine sediments and organic particulates it may be expected that this will affect the ecology and biology of both signal crayfish and native species. However, it has been shown that the signal crayfish is more tolerant to suspended solids and less susceptible to gill damage due to suspended solids than native species like WCC (Rosewarne *et al.*, 2014).

1.5.2 Impacts on macroinvertebrates

1.5.2.1 Impacts on native crayfishes

Non-native crayfish often serve as vectors of parasites and pathogens and can drastically reduce the abundance of native crayfishes (Lodge *et al.*, 2012). In the UK, severe negative impacts of signal crayfish on the native WCC is the prime concern in this regard (Freeman *et al.*, 2010). A range of studies showed the dominancy of signal crayfish over WCC. The invasive signal crayfish is harmful to the WCC, apparently due to its greater fecundity, more rapid development, larger size, aggressive behaviour and superior environmental tolerance capabilities (Lowery and Holdich, 1988; Peay, 2001; Bubb *et al.*, 2004). In addition, the signal crayfish (and several other North American crayfishes) is tolerant to, but spreads crayfish plague which causes mass mortalities of native European crayfish species. Crayfish plague is a fungal disease caused by the oomycete fungus, *A. astaci* (Alderman *et al.*, 1990) and the first report of its occurrence in Europe was in Italy in 1859 (Holdich, 2003). A combination of competitive superiority and adaptability, as well as the plague bearing capacity has made invasive signal crayfish one of the reasons for the decline of native crayfish species.

When signal crayfish establish themselves in a water body, the native population in downstream waters is exposed to crayfish plague due to transport of spores by flowing water (Frings *et al.*, 2013). The mortality rate from crayfish plague can be 100% in susceptible species (Oidtmann, 2000). Sometimes signal crayfish may temporarily co-exist with other native crayfish in a habitat (e.g. with WCC in River Shep, England, Slater *et al.*, 2000; with *A. astacus* in a lake of Finland, Westman and Savolainen, 2001) but later, outbreak of crayfish plague can lead to death of the native species, or in other cases native crayfish species are simply outcompeted and replaced over a timescale of about 3–10 years (Bubb *et al.*, 2006a).

Native WCC exhibit winter torpor in most parts of their range, including the UK (Brewis and Bowler, 1982). In contrast, signal crayfish exhibit reduced levels of activity at low temperatures but remain somewhat active and can exhibit substantial movement (> 100 m) during winter in northern England (Bubb *et al.*, 2002). No impact of high water flow on mortality or downstream displacement of signal crayfish was revealed in that study. A similar effect was seen in high flow during summer in another study of signal crayfish in an upland spate river (Bubb *et al.*, 2004). Unlike most non-winged stream invertebrates, crayfish are capable of substantial upstream as well as downstream dispersal (Bubb *et al.*, 2004). Tagging studies (Bubb *et al.*, 2004, 2006b) concluded that there is no apparent influence of size, sex or density on movement, although for young of the year (too small for tagging) most movement must be downstream (through drift). Adult signal crayfish move more than WCC but both use similar daytime refuges indicating likely competitive interaction between the two species (Bubb *et al.*, 2006a).

1.5.2.2 Impacts on other macroinvertebrates

In environments with high crayfish density there may be a shift in species composition of benthic macroinvertebrates towards active and sediment-burrowing taxa that are not dependent on macrophytes or vulnerable to direct predation from crayfish, observed in both lentic and lotic waters (Gamradt and Kats, 1996; Nyström, 1999; Crawford *et al.*, 2006; Ercoli *et al.*, 2015; Mathers *et al.*, 2016). Negative relationships

between herbivore / detritivore macroinvertebrate biomass or richness and crayfish abundance have been reported (Nyström *et al.*, 1996). Large invertebrates like freshwater pearl mussels (e.g. *Margaritifera laevis* and *M. togakushiensis*) are also reported to be eaten (those that are small in size) or injured by non-native signal crayfish and thus recruitment, growth and reproduction were hampered (Machida and Akiyama, 2013). Crayfish also have a strong ability to limit snail populations through predation (Dorn, 2013).

Invasive species often exhibit wider niche characteristics and environmental tolerance than native species and can be a threat to the conservation of threatened species (Mack *et al.*, 2000). This pattern is illustrated by negative impacts of invasive crayfishes on native endangered WCC in the UK (Bubb *et al.*, 2006a) and also on two endangered native freshwater pearl mussel species in Japan (Machida and Akiyama, 2013).

1.5.3 Impacts on macrophytes

Crayfish have been shown to have prominent grazing impacts, both direct and indirect, on aquatic macrophytes (Creed, 1994; Lodge *et al.*, 1994; Matthews and Reynolds, 1995; Nyström, 1999; Rodríguez *et al.*, 2003). Through direct consumption of macrophytes, coupled with their burrowing habits, invasive crayfish can effectively change a freshwater environment from macrophyte-dominated clear areas to phytoplankton dominated turbid areas (Rodríguez *et al.*, 2003; Geiger *et al.*, 2005; Matsuzaki *et al.*, 2009). Non-native signal crayfish can significantly reduce macrophyte biomass, cover and species richness in ponds (Nyström *et al.*, 1996) or experimental ditches (Roessink *et al.*, 2017). Within five years of establishment non-native crayfish can wipe out macrophyte cover almost completely from a closed water body, as recorded in a gravel pit lake of Germany (Gross, 2013). Similar complete loss of macrophytes is also reported in Swedish ponds as a result of signal crayfish invasion (Nyström *et al.*, 1996). Native crayfish can also affect macrophytes but the effects of non-native crayfish are usually stronger (Nyström *et al.*, 1999).

1.5.4 Impacts on amphibians

The impacts of invasive crayfish on vertebrates are less well documented than those on invertebrates and plants but studies have revealed that crayfish may feed on amphibian eggs and larvae (Reynolds, 1978; Ward and Sexton, 1981; Formanowicz and Brodie, 1982). Several studies have shown that the spread of non-native crayfish into new habitats can have negative effects on amphibian populations, generated principally through decreased egg and larval survivorship (Nyström, 1999; Kats and Ferrer, 2003). Impacts of non-native red swamp crayfish *P. clarkii* on California newt *Taricha torosa* have been reported to decrease densities of adult newts, larvae and their egg masses significantly in newly invaded areas by crayfish (Gamradt *et al.*, 1997). Newt individuals also suffered from physical injuries from direct attacks by crayfish (Gamradt *et al.*, 1997). Eggs and larvae of California newt were also predated by introduced crayfish leading to a situation where no larvae can survive (Gamradt and Kats, 1996). Similar predation by signal crayfish on eggs and larvae of seven species of amphibians (frogs and toads) were also reported in Sweden through aquarium and pool experiments (Axelsson *et al.*, 1995). However, non-native crayfish can be a more effective amphibian predator than native crayfish (Renai and Gherardi, 2004).

1.5.5 Impacts on fishes

Crayfish can potentially have negative effects on fish through direct predation of eggs, larvae and small adults, but also through competition for food and shelter and by destroying breeding sites (e.g. macrophyte reduction). Laboratory experiments revealed that invasive crayfish, particularly the larger individuals, can prey on unburied fish eggs and may be a threat to salmon (Edmonds *et al.*, 2011; Findlay *et al.*, 2015). Although Gladman *et al.* (2012) found signal crayfish did not present a threat to Atlantic salmon *Salmo salar* via predation of buried eggs in laboratory conditions, Edmonds *et al.* (2011) did find evidence of capture and predation of salmon alevins (newly hatched young with yolk sacs, still in the gravel) and fry. Findlay *et al.* (2015) demonstrated that juvenile crayfish are small enough to pass through the interstices of salmon redds and can damage and eat salmon eggs. In the wild, a negative relationship between

invasive signal crayfish and sea trout and Atlantic salmon abundance was reported in a headwater stream of northeast England (Peay *et al.*, 2009). The study also reported a major decline in salmonids over time in relation to increasing abundance of signal crayfish.

Populations of small benthic fish (e.g. bullhead) can also be affected adversely by invasive crayfish. Under laboratory conditions with refuges signal crayfish make aggressive approaches towards bullhead (Bubb *et al.*, 2009) and since crayfish use the same refuge habitat and are dominant to bullhead, they are likely to access and eat bullhead eggs laid on the undersides of boulders (Findlay, 2013). During reproduction male bullhead build nests and guard the fertilized eggs (Marconato *et al.*, 1993). A negative relationship between benthic fish density and signal crayfish density has been observed in field surveys (Guan and Wiles, 1997; Bubb *et al.*, 2009). Laboratory experiments show that signal crayfish are able to displace both benthic fish and juvenile salmonids from their shelters (Rahel and Stein, 1988; Griffiths *et al.*, 2004; Light, 2005; Bubb *et al.*, 2009). This crayfish-induced eviction of fish from shelters may provoke their susceptibility to predation by crayfish and other species, especially birds, mammals and fish (Taylor, 2002; Bubb *et al.*, 2009). Similarly, the reduction of cover in the form of aquatic macrophytes by crayfish may indirectly affect fish assemblages and abundance through increasing their vulnerability to predation.

It is not always the case that the fish community is affected by signal crayfish. A study based on 61 temperate streams in Sweden revealed no significant changes in fish communities as a whole in the presence of crayfish, including signal crayfish (Degerman *et al.*, 2007). This seems quite unlikely, but possibly environmental variables were dominant in determining community structure or the monitoring design or method was insufficiently sensitive for detecting change. However, our knowledge regarding specific ecological impacts and the mechanisms behind them are limited for most invaders (Jackson *et al.*, 2014). The outcome of a particular introduction of a non-native species cannot easily be predicted because such responses are affected by a large number of factors and thus, it is essential to study the potential invader along with target community

thoroughly (Lodge, 1993). Though several studies have been carried out separately in order to determine the impact of signal or other crayfish on specific community elements, further study of this issue is warranted especially with a view to assessing the impacts of invasive crayfish on vertebrates, especially indigenous fishes.

To better understand the degree of competitive interaction between invasive signal crayfish and other native species it is important to study the structure and dynamics of their habitats. Use of stable isotopes to evaluate the trophic structure and dynamics of ecological communities is now a well-accepted method for this purpose (Middelburg, 2014; Perkins *et al.*, 2014). One of the advantages of this technique is that it combines benefits of both the trophic level and food web paradigms in food web ecology (Post, 2002). Carbon stable isotopes ($\delta^{13}C$) are used to investigate the structure of food webs by determining the sources of carbon and its vertical movement within the food web (predator/prey interactions) (Post, 2002). In conjunction with analysis of nitrogen isotopes ($\delta^{15}N$), researchers are able to determine key characteristics of food webs such as trophic position, food chain length and level of omnivory (Post, 2002; Dodd, 2010). As a result, this method quantifies the seasonal, temporal and spatial heterogeneity of ecosystems and allows the examination of spatial extent and long-term dynamics of food webs.

In addition to conventional methods based studies, several stable isotope studies (e.g. Bondar *et al.*, 2005; Jackson *et al.*, 2014; Wood *et al.*, 2017) have been carried out in order to understand the trophic position of signal crayfish in different environments, but not in upland streams. A recent research study, using lentic mesocosms was conducted in the lowland Thames catchment by Jackson *et al.* (2014), while Bondar *et al.* (2005) explored the effects of ontogenetic stage and density on food choices. Neither included any potential competitor indigenous species and no such study has been conducted in upland rivers. Moreover, less attention is given to the density dependent impacts of invasive crayfish that could be an important driver for regulating trophic interactions in the ecosystem (Ludlam *et al.*, 2015). All these issues are addressed in this thesis (Chapter Two) and thus, the outcome would provide useful

information (e.g. distribution and population density) to the development of a regional inventory of alien species recommended by European Strategy on Invasive Alien Species (Council of Europe, 2002).

Existing studies are mostly short term or conducted in controlled laboratory environments which may not be appropriate for predicting the impacts of crayfish in the wild where many ecological or other factors are present and can influence the impacts of a species (Degerman *et al.*, 2007). In field studies it is difficult to determine factors, including invasive crayfish, responsible for changes in fish populations, without controlling for habitat and year-to-year recruitment variability, and this issue has not been fully addressed (Degerman *et al.*, 2007; Peay *et al.*, 2009). Moreover, a before-after-control-impact (BACI) method has rarely been employed in existing studies which is more accurate in quantifying changes in population of target species. Chapter Four of this thesis addresses these issues through a study of medium to long-term impacts of signal crayfish on macroinvertebrates and native fish populations in upland steams of northeast England. The study employs a BACI method and consideration of the influence of major physico-chemical parameters of water and habitats.

A three-stage hierarchical approach to management of alien invasive species has been suggested by the Convention on Biological Diversity (CBD) and these are (i) prevention, (ii) surveillance and rapid response, and (iii) control and eradication (Roy *et al.*, 2014). But, there is lack of a comprehensive regulatory framework of invasive species (Caffrey *et al.*, 2014). Although various control methods have been considered to stop or slow down invasive crayfish invasion (Gherardi, Aquiloni, *et al.*, 2011) it is evident that no single method can yield a desirable solution to the problem (Freeman *et al.*, 2010). Therefore further studies are needed in this regard, with a view to improving the performances of existing methods or inventing a new method that will be more effective. Dispersal and colonisation of invasive species is a key factor in understanding the replacement of inferior competitors and thus, study on the potential factors that drive the invasion dynamics of a species may be important, especially for management plans for controlling an invasive species. In recent times, animal personality is being studied to understand different aspects of

biological invasion including dispersal (e.g. Duckworth and Badyaev, 2007; Cote, Clobert, *et al.*, 2010; Cote, Fogarty, *et al.*, 2010). It has been suggested that animal personality should be incorporated to any management plans to increase its efficiency, especially for controlling a non-native species (Juette *et al.*, 2014). There is not much research on personality of an invasive species. A few available studies (Duckworth and Badyaev, 2007; Cote, Fogarty, *et al.*, 2010; Malange *et al.*, 2016) have suggested that the dispersal of a non-native species is linked to the personality traits of individuals and a personality biased population may be expected at the invasion front. In animal personality studies, bolder individuals are expected to be more exploratory and successful in resource exploitation (Sundström *et al.*, 2004; Ward *et al.*, 2004; Smith and Blumstein, 2008) and thus may play a key role in the range expansion of the species. However, many of these studies have been laboratory based and none of these studies considered other potential ecological factors that could also affect the invasion process. Therefore, research is required in this regard to better understand the role of personality in invasive animal dispersal in the wild. Chapter Three of this thesis will present the outcomes of a personality-dependent signal crayfish dispersal study, with consideration of other potentially influencing biological and ecological factors in two upland streams. The findings of this study may contribute significant knowledge towards better management of invasive crayfish.

1.6 Focal species in this research

In this research the signal crayfish *Pacifastacus leniusculus* (Figure 1.3) was used as a model AIS. Crayfishes, particularly signal crayfish, are among the most successful and widely distributed invasive animal species (Holdich *et al.*, 2014) thus an excellent organism to study as a model species of invasive nature. Moreover, though significant focus has been given to biological invasion by this species there is still a gap in knowledge regarding invasion ecology of invertebrate taxa (e.g. lack of information about the extent of distribution of taxa, processes driving invasion, and mechanisms of invasion impacts, especially in inland waters; Gherardi *et al.*, 2009), including this species.

The signal crayfish is endemic to western North America (Lewis, 2002), west of the Rocky Mountains (Hobbs, 1988). This species is also the most widespread of all the introduced crayfish in Europe (Bubb *et al.*, 2004), colonising over 20 countries on this continent since the 1960s (Figure 1.4). This species was introduced to Europe as an aquaculture species from the 1960s onwards as a replacement species for local crayfish species, particularly for the noble crayfish *Astacus astacus* in Sweden, after the spread of crayfish plague and decline of native species in Europe (Ibbotson and Furse, 1995). The signal crayfish has now established wild populations in most northern European countries because of escape from aquaculture farms and deliberate introductions (Lowery and Holdich, 1988; Holdich, 1999). However, although signal crayfish were introduced to Europe for farming, production levels were quite low (Holdich, 1993). In the UK, signal crayfish were legally introduced in 1976 for the purpose of aquaculture (Lowery and Holdich, 1988; Peay and Rogers, 1999). Within about a decade of introduction, by 1988, this species had colonized more than 250 water bodies in the UK (Lowery and Holdich, 1988).

FIGURE 1.3 Non-native American signal crayfish *Pacifastacus leniusculus*, caught from Wilden Beck, a tributary of the River Tees.

Signal crayfish have been recorded to have impacts on a wide range of native species of conservation interest including native crayfishes, fishes and non-crayfish invertebrates (Bubb *et al.*, 2006a; Machida and Akiyama, 2013). In this study, a main target species for conservation was the small benthic bullhead (*Cottus perifretum* = *C. gobio* in the UK, Cottidae, a member of the unresolved *Cottus* species complex) and other indigenous finfish species (e.g. brown trout *Salmo trutta*, Atlantic salmon *Salmo salar*, minnow *Phoxinus phoxinus*, stone loach *Barbatula barbatula*, three-spined stickleback *Gasterosteus aculeatus* and others) in upland rivers of the UK. Signal crayfish have been implicated in impacting benthic fishes (Guan and Wiles, 1997; Bubb *et al.*, 2009) and salmonids (Edmonds *et al.*, 2011; Findlay *et al.*, 2015), but evidence remains incomplete. Conservation and population status of bullheads are 'Least Concerned' and 'Unknown' respectively on the IUCN Redlist database (Freyhof and Kottelat, 2016). Cottids are important benthic fishes in streams and can play important roles in ecosystems, often being more abundant than species of commercial or angling importance (e.g. brown trout; Mills and Mann, 1983). In the UK the bullhead is a Biodiversity Action Priority speces, but is itself listed on the EU Habitats and Species Directive, but itself a non-native invader in Scotland.

Although an initial aim of this study was to include, in addition to existing native fishes, comparative studies of the effects of native white-clawed crayfish (WCC) *Austropotamobius pallipes* this was not possible because of the lack, locally of parapatric populations of signal crayfish and white-clawed crayfish within the same river, and difficulties in securing a licence for the studies planned.

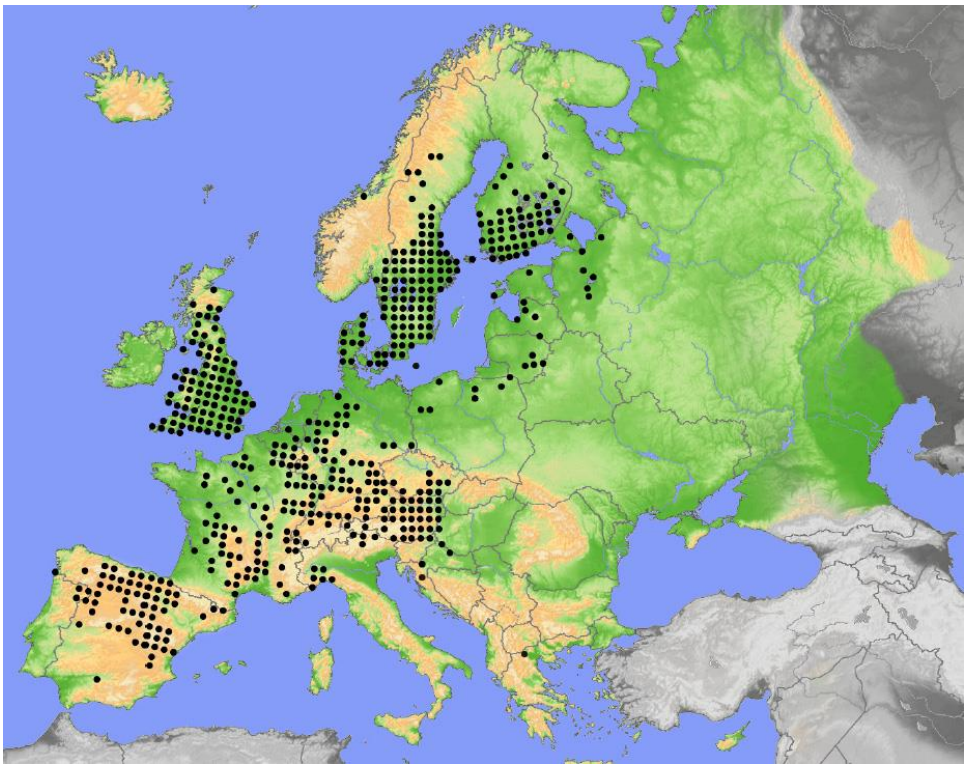


FIGURE 1.4 Distribution of signal crayfish *Pacifastacus leniusculus* in Europe, presence in 50 × 50 grid squares is represented by dots (source: Kouba *et al.*, 2014).

1.7 Aims and objectives of the study

The major aim of this study is to measure the impacts of non-native crayfish as a prominent example of an invasive species mediating ecosystem disruption and its various component alterations. This study aims to determine the mechanisms affecting direct and indirect effects of invasive signal crayfish on upland stream biodiversity and on key native species of conservation and fisheries importance. The study involved comparisons of these effects, especially the ecological effects of signal crayfish in streams, to those without signal crayfish. From the foregoing and studies on dispersal and colonisation of invasive crayfish in relation to practices of stream connectivity restoration, this study would contribute to the improvement of conservation planning strategies to reduce invasive impacts in streams and to support conservation of native stream biodiversity.

This study tests the following hypotheses concerning our understanding of the ecological impacts of invasive signal crayfish as key factors affecting aquatic ecosystem alteration impacting biodiversity conservation and economic value:

- **Hypothesis 1:** Invasive crayfishes exert strong effects on stream communities in terms of community structure, biodiversity, productivity and food webs.

- **Hypothesis 2:** The bold, aggressive behaviour of signal crayfishes, their ability to attain high densities and their benthic habits are responsible for reduction in populations of benthic fishes.

- **Hypothesis 3:** Bold crayfish are those responsible for initiating and maintaining an 'invasion front'.

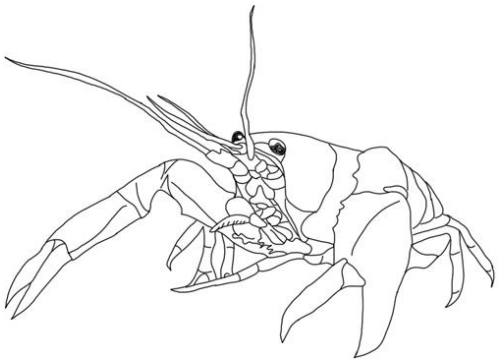
- **Hypothesis 4:** Loss of native species and reduction in stream biodiversity in response to colonisation by invasive crayfish represents a long-term ecological phase shift and chronic induced ecosystem alteration, rather than a transient pulse of invasion abundance and community flux.

1.8 Chapter outlines

Following the General Introduction this thesis comprises three data chapters (Chapters Two, Three and Four) and a General Discussion (Chapter Five). Chapter Two, aimed at addressing hypotheses 1 and 2, is based on a mesocosm study in the River Lune of northeast England in which impacts of signal crayfish, at different densities, were determined on native small benthic fish (bullhead), invertebrate communities, algal primary production, leaf litter decomposition and trophic structure. In addition to quantification of changes in these animals / groups over time, stable isotope analysis was also employed in this study to identify the functional role of signal crayfish and to understand the flow of energy and trophic interactions in the community.

Chapter Three is based on a study that measures dispersal of signal crayfish in invaded streams in relation to their behavioural, biological and ecological factors. This chapter addresses hypothesis 3, aimed at understanding invasion dynamics of a non-native species. Outcomes of this study might be helpful for better understanding the invasion dynamics of signal crayfish in natural environments. This study was carried out with three crayfish sites (fully-established, newly-established and invasion front) across two upland streams of northeast England.

Chapter Four, aimed at addressing hypotheses 1, 2 and 4, particularly the latter, described a study that shows moderate (7 years) and long-term (since 1990) impacts of signal crayfish on indigenous fish and macroinvertebrate communities in different upland streams of the River Tees, northeast England. This study measures impacts of signal crayfish on native fish populations, especially on the benthic fishes and salmonids (brown trout and Atlantic salmon) and macroinvertebrate communities over time and also complements Chapter Two and *vice-versa*. Lastly, Chapter Five summarises the key findings of the study, sets it into a broader invasive species management context, and considers the future for upland stream ecosystems and native biota conservation in catchments invaded by non-native crayfishes.



Chapter Two

UNDERSTANDING DENSITY-DEPENDENT IMPACTS OF SIGNAL CRAYFISH ON STREAM ECOSYSTEMS: A MESOCOSM APPROACH

Summary

The nature and extent of non-native species ecosystem impacts, including those of invasive crayfishes, may be density-dependent, but these have received less attention than presence-absence effects. In this mesocosm-based study, conducted in the River Lune, NE England, density-dependent impacts of invasive signal crayfish *Pacifastacus leniusculus* on different ecosystem components were assessed. The experiment involved two control (C_1, without fish or crayfish; C_2, native fish [bullhead *Cottus gobio*] only) and three treatment (T_1–T_3, with a fixed density of bullhead and varying densities of crayfish) groups, each with five 1.5m^2 enclosure replicates over a period of 47 days during summer.

Strong impacts of crayfish at all three densities on macroinvertebrate (density, taxonomic composition), native fish (growth, diet) and ecosystem processes were recorded. Despite similar invertebrate abundance and richness across enclosures before introducing crayfish and bullhead, they varied significantly from controls at the end, with >80% reduction in macroinvertebrate abundance recorded in T_3. Stable isotope ($\delta^{15}N$ and $\delta^{13}C$) analysis showed that the trophic niche of signal crayfish did not change in sympatry with bullhead, but that of bullhead did. Bullhead in treatment enclosures consumed a reduced proportion (by ~5–10%) of macroinvertebrate larvae occupying higher position in food web than those from control group. Bullhead in T_3 lost 4.2% of the initial weight over the study period. Ecosystem processes were affected by crayfish density. Leaf litter break down was faster, but algal standing crop was lower, in treatments with greater densities of crayfish.

This study concludes that signal crayfish, even at a low density, can strongly impact multiple ecosystem components and can efficiently play the role of an ecosystem engineer or a keystone species which may result in a strong trophic cascade.

Keywords: Enclosure-exclosure, trophic niche, trophic cascade

2.1 Introduction

Biological invasion of non-native species is currently one of the major global threats to world's biodiversity (Lodge, 1993; Naeem *et al.*, 2012; Caffrey *et al.*, 2014; Veale *et al.*, 2015). Invasive species can affect native species and ecosystems in different ways, directly and / or indirectly, including by alteration of food web structure, by decreasing species richness and the number of links per species (Gherardi *et al.*, 2009; Galiana *et al.*, 2014). In doing so they pose a threat to ecosystem integrity and functioning.

The impact of invasive crayfishes on native fauna and flora is well-documented (e.g. Edmonds *et al.*, 2011; Ruokonen *et al.*, 2014; Findlay *et al.*, 2015) but controlled studies in lotic ecosystems are rare. Impacts on amphibians have been recorded for several crayfish invasive taxa including *Procambarus clarkii* (Axelsson *et al.*, 1995; Gamradt and Kats, 1996; Gamradt *et al.*, 1997). Invasive species often exhibit wider niche characteristics (i.e. the capability of exploiting varieties of resources in a habitat; Roughgarden, 1972) and environmental tolerance than native species and can be a threat to the conservation of threatened species (Mack *et al.*, 2000). This pattern is illustrated by impacts of invasive crayfishes on white-clawed crayfish (WCC) *Austropotamobius pallipes* (Bubb *et al.*, 2006a) and also on two endangered native freshwater pearl mussel species (*Margaritifera laevis* and *M. togakushiensis*) (Machida and Akiyama, 2013). Thus, in recipient habitats invasive crayfish, including signal crayfish – one of the most recognised invasive crayfishes in different parts of the world for its adverse impacts on the native biota (e.g. Stenroth and Nystrom, 2003; Bubb *et al.*, 2009), can be a serious threat to native fauna. However, the impacts of an omnivorous species like signal crayfish on native communities are difficult to predict due to their broad diets, behavioural flexibility and diverse abiotic and biotic factors in invaded habitats (Klose and Cooper, 2012).

Apart from direct impacts, an invasive species can also exert strong effects on recipient ecosystems through the process of trophic cascade whereby a consumer affects non-adjacent trophic levels through alteration

of prey abundance and behaviour and results in an indirect effect on subsequent trophic levels (Hairston *et al.*, 1960; Threlkeld, 1988; Silliman and Angelini, 2012). In an invaded habitat invasive species can create new trophic links and can also modify or disrupt existing ones (Jackson *et al.*, 2017). Effects of trophic cascade can be severe on aquatic ecosystems (Carpenter *et al.*, 1985; Carpenter and Kitchell, 1988; Strong, 1992; Ousterhout *et al.*, 2018). Thus, to understand the degree of competitive interaction between invasive signal crayfish and other native species (e.g. WCC, bullhead and others) it is important to study the use of habitats and key resources such as food. Although many studies have focused on impacts of invasive crayfish on native biota, less attention has been given to the density-dependent impacts of crayfish which may be an important factor in determining its role in invaded ecosystems, especially if density alters the strength of trophic interactions (Ludlam *et al.*, 2015).

Stable isotopes, typically carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), can represent an organism's trophic niche and are widely used to examine aspects of food web structure (Post, 2002; Layman *et al.*, 2007). These two stable isotopes are commonly used to trace organic matter through food webs (McCutchan and Lewis, 2001). The structure of food webs by determining the sources of carbon can be investigated by analysing $\delta^{13}C$ isotope (Post, 2002) whereas $\delta^{15}N$ are used to study vertical movement within the food web (predator/prey interactions) (Post, 2002; Layman *et al.*, 2007). However, isotopic shift (fractionation) between a consumer animal and its diets is usually small for carbon, less than 1‰ (DeNiro and Epstein, 1978; Fry and Arnold, 1982; Peterson and Howarth, 1987). On the other hand, for nitrogen, fractionation is larger, usually 1 – 5‰ (DeNiro and Epstein, 1981; Minagawa and Wada, 1984). Moreover, influences of invasive species on different critical ecosystem processes (e.g. primary / algal production and leaf litter decomposition) can also provide important information regarding our understanding of trophic cascades within a habitat (Moore *et al.*, 2004; Woodward *et al.*, 2008). This is particularly important in many food webs fuelled by both autochthonous production and allochthonous detrital subsidies (Moore *et al.*, 2004) and it is important to

address an animal's impacts on both energy sources while assessing its role in an ecosystem (Woodward *et al.*, 2008).

Several stable isotope studies (e.g. Bondar *et al.*, 2005; Stenroth *et al.*, 2006; Jackson *et al.*, 2014; Wood *et al.*, 2017) have been carried out in order to understand the trophic position of signal crayfish in different environments, but not in upland rivers. Recent research was conducted in the lowland Thames catchment by Jackson *et al.* (2014) to show interactions between signal crayfish and other non-native crayfish species, while (Bondar *et al.*, 2005) explored the effects of ontogenetic stage and density on food choices. However, studies to reveal the interactions of signal crayfish with finfishes are scant. Effects of signal crayfish on growth, diet, and trophic position of a native fish species, chub (*Squalius cephalus*) were recently published (Wood *et al.*, 2017). Chub is an omnivorous species, somewhat similar in this regard to signal crayfish, but occupies a different habitat niche (chub is a highly mobile, midwater animal, reaching a much larger body size than signal crayfish, but relying on gravel-bottomed rivers for reproduction; Freyhof and Kottelat, 2007). Thus it can be expected that the species occupying the same niche and of similar habits (e.g. uses refuge as shelter) to signal crayfish are more susceptible to signal crayfish invasion.

Bullhead (*Cottus gobio*) is native to England and is a small (usually < 10 cm) bottom-dwelling species (i.e. benthic) (Freyhof and Kottelat, 2008). Under controlled conditions it has been shown that signal crayfish can outcompete bullhead for shelters and if refuges are a rare resource signal crayfish tend to oust bullhead from these (Bubb *et al.*, 2009). A negative relationship between signal crayfish and bullhead abundances has been recorded in the wild (Guan and Wiles, 1997; Bubb *et al.*, 2009), potentially due to reduced bullhead survival in sympatry with signal crayfish. Moreover, both bullhead and signal crayfish feed on common diets including macroinvertebrates (Dahl, 1998; Stenroth and Nyström, 2003). So, density-dependent impacts of signal crayfish on diet and growth of bullhead or other benthic fishes can be hypothesized. This study would also contribute to an improved understanding of competitive mechanisms in benthic animals.

The mesocosm approach, used in this study, is a common and well-established method in ecological research to understand the impact of a particular animal species on the target species or community (e.g. Greig *et al.*, 2013; Schwindt *et al.*, 2014; Tran *et al.*, 2015). Several mesocosm-based studies of crayfishes have evaluated impacts of red swamp crayfish (*Procambarus clarkii*) on macrophytes, macro crustaceans and macroinvertebrates (Rodríguez-Pérez *et al.*, 2016); white-clawed crayfish (*Austropotamobius pallipes*) on invertebrates (Rosewarne *et al.*, 2013); interaction among different non-native crayfish species (signal, *P. leniusculus*; virile crayfish, *Orconectes virilis*; red swamp crayfish, *P. clarkii*; and Turkish crayfish, *Astacus leptodactylus*) and their impacts on benthic invertebrate communities (Jackson *et al.*, 2014).

In this study, the impact of non-native signal crayfish on macroinvertebrates, native fish species and ecosystem processes (e.g. leaf litter decomposition and algal standing crop) were measured through mesocosm experiments. Replicated enclosures were used with varying densities of signal crayfish and a fixed density of bullhead. This experiment provides a classical ecological design for teasing apart the influence of different factors.

The study sought to test the following hypotheses:

1. Signal crayfish exert strong effects on stream communities in terms of community structure, biomass and food webs. It was predicted that these effects on different ecosystem components would be stronger with increasing density of signal crayfish.
2. Signal crayfish impact native benthic fish through interference competition, reducing their growth through food web alteration. Thus, density-dependent responses in trophic niche and growth of bullhead were predicted in response to varying densities of signal crayfish.

2.2 Materials and methods

2.2.1 Study sites

This study was conducted in the River Lune, a tributary of River Tees near Mickleton, County Durham in the north east of England (**Figure 2.1**). The study location was located at the downstream end of the River Lune near the Lune–Tees confluence between 54°37'09.6"N 2°03'19.8"W and 54°37'13.0"N 2°03'09.4"W), about 3.5 km downstream of Grassholme Reservoir. Site selection was conducted during summer 2017.

FIGURE 2.1 Map of the River Lune showing the study area (blocks A to E) for the current experiment. Enclosures are represented by yellow dots within the stream reach (map is modified from Digimap).

The chosen study site needed to be within a viable daily travelling distance of Durham for intensive fieldwork and required a location where enclosures would not be disturbed. It also necessitated an unpolluted site with an established population of signal crayfish in sympatry with native finfish including bullhead and the presence of various invertebrates (e.g. mayfly larvae and caddis fly larvae). It also needed to have suitable natural substrate, comprising a variety of particle sizes, especially including cobbles and boulders as refuges for crayfish and benthic fishes, suitable water depth and a stable flow of water over the entire experimental period. The site needed to be large enough to enable the secure in-channel erection of up to about 25 enclosures without excessive flow alteration or channel obstruction and needed to have the hydrological stability for a low probability of damage or washout due to high flows. Such hydrological

conditions are most common in spring-fed streams or on regulated mill streams, but these are quite rare in the region, and in any case tend to be too narrow for the scale of the planned enclosures. Although most running waters below impoundments in NE England (e.g. the Lune, about 1 km below Grassholme Reservoir) are subject to spates in winter during reservoir overtopping, this is much rarer in summer, the planned season for fieldwork (the main growth and activity period of ectotherms including crayfish and finfish). It was also expected that the study site would be free from water pollution and risk of interference from the public.

During site selection a combination of hand-net searching (half an hour with two people searching potential refuges at each site, thus equivalent to one person-hour) and electrofishing were carried out at different spots of the River Lune in order to assess the presence of finfishes, signal crayfish and other invertebrates. The site identified comprises a reach of approximately 350 m comprising slow glide, faster glide and riffle localities with natural substrate and is mostly ~10 m wide and mostly ~0.2 – 0.5 m deep at base flows. In September 2017 bullhead and trout were abundant, minnow were locally abundant and salmon juveniles were present. Signal crayfish were abundant, with a wide variety of size classes evident. Signal crayfish are known to have colonised Grassholme Reservoir upstream, possibly as a result of direct introduction as the reservoir has a 25-m high dam, likely preventing colonisation in an upstream direction, from the Tees. Colonisation of the lower Lune by signal crayfish has likely been over the last 10–20 years as Findlay (2013) found none in the lower Lune at a site that overlaps with the study area of the present research and all records in Grassholme seem to be since 2010 (Environment Agency, unpublished data). White-clawed crayfish (*A. pallipes*) have not been recorded in the Lune subcatchment for several decades and are now only known in the Tees catchment from a small handful of sites, including a small tributary of the Balder (which meets the Tees ~8 km downstream of Lune-Tees confluence) and some water supply reservoirs in the lower Tees catchment. Experiments were carried out under DEFRA permit to M. Lucas, and Home Office and institutional animal welfare committee permissions to M. Lucas.

2.2.2 Methods

2.2.2.1 Experimental design

A total of twenty five enclosures (each 1.5 m long × 1 m wide × 0.7 m height = 1.5 m^2 area) were used in this study (Figure 2.2). Enclosures were constructed with wooden frame and heavy-duty plastic mesh nets, each with a mesh lid, cable-tied shut. The mesh size (5 × 5 mm) of net used in this study was large enough to ensure movement and colonisation by small (and juveniles of) invertebrate species but not crayfish and fish. Before the experiment a pilot study with three enclosures (Enclosures A–C) was carried out for 20 days from 24 May 2018 to 12 June 2018. This was carried out in order to refine the design and installation of enclosures and check their stability against the river flow variation and efficiency of holding fish and signal crayfish (for details of enclosure instalment see the following section 2.2.2.2). These operated successfully.

FIGURE 2.2: A block containing five enclosures in the River Lune.

The main experiment was conducted between 20 June and 31 August, 2018 that involved time for enclosure conditioning (first 27 days, until 16 July 2018; to allow macroinvertebrate colonisation) and study with fish and / or signal crayfish in the enclosures (47 days, from 16 July 2018). Five different combination groups including two controls (C_1, without native fish or signal crayfish; C_2, native fish only) and three treatments (T_1 – T_3; with varying densities of signal crayfish and a fixed density of native benthic

species, i.e. bullhead) were employed (see Table 2.1 for details of groups and densities used). In each treatment/control five replicates (R_1 – R_5) were used.

TABLE 2.1 Study design for the current experiment; species density represents number per 1.5 m^2 area.

Groups	Type	Species combinations	Density	
			Bullhead	Crayfish
C_1	Control	Without fish or crayfish	0	0
C_2	Control	Bullhead only	5	0
T_1	Treatment (Low)	Bullhead + signal crayfish	5	4
T_2	Treatment (Mod)	Bullhead + signal crayfish	5	8
T_3	Treatment (High)	Bullhead + signal crayfish	5	12

For signal crayfish (25 – 30 mm carapace length [CL]), experimental densities covered a range observed in rivers and their tributaries in north east England (based on field surveys conducted in summer 2017 in which a maximum density of 12.82 crayfish per m^2 was recorded). Equal numbers of male and female crayfish were used in each enclosure to nullify the possibility of any sex-biased outcomes. No females were carrying eggs/young; the timing of the study was after all females had released young (S. Galib, pers. obs.). Bullhead density was chosen according to the natural density in UK rivers, reported in different studies (Table S2.1 in Appendix I). Findlay (2013) reported a local density of 0.1 – 2.4 individuals m^{-2} in different tributaries of the River Tees including the River Lune of this study although higher densities have been reported in the River Tees (up to 5.2 individuals m^{-2}, Mills and Mann, 1983). Thus five individuals were used in each enclosure in this experiment. Benthic fish and signal crayfish were introduced on 16 July 2018 (see section 2.2.2.3 for collection of fish and crayfish).

2.2.2.2 Enclosures set up

The enclosures were manually dug into the streambed to a depth of about 30 cm and 1.5-m holding stakes driven in place at each corner. Enclosures were then refilled, covering the bottom mesh with substrate to a depth that

matched the level outside. Enclosures were allowed to condition for about four weeks to facilitate natural algal growth and macroinvertebrate colonisation prior to the introduction of fish or crayfish to the enclosures. All control and treatment groups were replicated in five randomised complete blocks (block IDs: A–E, each about 30 m long; Figure 2.1), installed within a ~250 m stretch of the river. Positions of enclosures belonging to different groups was assigned randomly within each block.

Prior to setting up enclosures, substrate characteristics at the site were recorded by counting and measuring boulders (>256 mm), cobbles (64 – 256 mm) and pebbles (16 – 64 mm) (following a simplified version of the Wentworth Scale; Wentworth, 1922) using a 1 m × 1 m quadrat (N = 20). At this site, finer sediments (< 16 mm, gravel, sand, silt) were incidental and mainly occurred in pockets within the larger sediment interstices. The mean number of larger substrate particles, i.e. boulders and cobbles, and their size (area) were calculated per m^2 quadrat. Based upon this, equal numbers of larger particles of similar sizes (boulders, n = 4, mean area 559 cm^2; cobbles, n = 78, mean area 124 cm^2; pebbles, n = 50, mean area 16 cm^2) were used to refill every enclosure. Approximately equal amounts of smaller substrates (i.e. gravel and smaller substrates, total ~5000 ml) were also added. This ensured similar shelter opportunities within the enclosures for study animals to those of outside habitat per unit area. Substrate particle volume and composition may have differed to a small extent across enclosures but careful attempts were made to minimise variations.

Mesh lids, shut tightly with cable ties, were employed on the top of each enclosure to make sure that crayfish or fish could not escape or enter through the top. The lid was 0.1 – 0.2 m above the normal water surface, depending on the natural gradient of the river, to minimise the chance of fish escape during checking of the enclosure. The heavy duty plastic mesh used was aimed at minimising the probability of enclosure damage due to abrasion by substrate, and resultant escape of study animals. During the experiment, the sides and tops of the enclosures were brushed biweekly to prevent debris build up and maintain flow through the enclosure.

2.2.2.3 Collection of signal crayfish and bullhead and individual marking

Signal crayfish and bullhead used in the experimental enclosures were collected from the River Lune in and around (within 200 m) of the study site where they exist in sympatry. Some of the planned components of this study (e.g. stable isotope analyses; see section 2.2.2.6 below) may be affected by animals if animals are collected from outside of study habitat. This is due to potential slow turnover rate of crayfish or bullhead tissues as the influence of previous diet on stable isotope ratios can be long lasting (McCutchan *et al.*, 2003).

Bullhead were collected by electrofishing (using a land-based generator, Honda EU inverter 10i; and an electrofishing control unit, model Electracatch WFC4, Electracatch International, Wolverhampton, England). Captured bullhead individuals were kept at a very low density in semi-transparent plastic tanks (at two individuals per tub with shelters; tank size: 35 cm long × 21 cm wide × 21 cm high) in shade, filled with river water, until further processing on the same day. Only similar sized bullhead (70.4 ± 3.6 mm; 4.4 ± 0.8 g; LMM, $P > 0.05$ across groups) were used in this study to avoid any size and biomass-biased results. Total length (mm) and body mass of bullhead were measured using a Vernier slide calipers (to the nearest 0.1 mm) and a standard pan balance (to the nearest 0.001 g) respectively. After measurements, bullhead were sedated in buffered tricaine methansulphonate (0.1 g L^{-1}, using river water) and individually marked to determine changes in individual length and weight at the end of the experiment by using Visible Implant Elastomer (VIE; Northwest Marine Technology, Inc., Shaw Island, WA, USA) tags, coded by mark location on the ventral side, and kept in the plastic tubs again for further observations. After about one hour, they were checked again (all behaving normally) and introduced to the enclosures.

Signal crayfish were caught by hand-net searching from the river and kept in plastic tanks at a low density (three crayfish per tank with shelters) until further processing, outlined above. Carapace length of crayfish (CL, length from rostral apex to the posterior median edge of the

carapace; Brewis and Bowler, 1982) and weight were recorded using the same instruments described above. Sex and any obvious marks on the crayfish's body (e.g. leg loss or other body marks) were also noted. Following physical examinations, crayfish were marked individually by VIE, coded by mark location on abdominal somites. VIEs are an effective tagging technique for both adult and juvenile crayfish that perform well without affecting crayfish biology, and are retained after moulting (Clark and Kershner, 2006).

2.2.2.4 Macroinvertebrate sampling

Two weeks before introducing fish and signal crayfish to the enclosures (i.e. almost two weeks after enclosure deployment in the river) macroinvertebrate samples were collected from each of the 25 enclosures (N = 3 from each enclosure) using a 0.1 m^2 Surber sampler in order to determine macroinvertebrate taxonomic richness, abundance and community structure before introducing study animals to the enclosures. Macroinvertebrate samples were collected again, by the same method, on the final day of experiment in order to determine changes (i.e. effects of signal crayfish or bullhead) in macroinvertebrate richness, abundance and community.

On both macroinvertebrate sampling occasions, immediately after collection samples were preserved in labelled jars using 70% ethanol solution and brought back to the laboratory for identification. The samples were identified under a low power microscope (Zeiss, Germany), using morphometric characteristics and following standard literature (Macan, 1959; Hynes, 1977; Croft, 1986; Wallace *et al.*, 1990; Edington and Hildrew, 1995; Pawley, 2011).

2.2.2.5 Recapture of signal crayfish and bullhead

On the final day of the enclosure experiment signal crayfish and bullhead were collected from all enclosures. They were counted, identified by VIE marks and length and weight were measured by the same method and equipment as in section 2.2.2.3. Individuals were carefully examined for any obvious signs on the body (e.g. wound marks on bullhead or claw / leg

loss of crayfish). All signal crayfish (*N* = 120) and a proportion of bullhead (60%, *N* = 60) were then euthanized in MS222, transferred to a cooled box with ice and brought back to the laboratory for stable isotope analysis (see below, section 2.2.2.6) and stored at –20°C. Body weight gain was calculated for each bullhead by deducting initial weight (i.e. weight at the start of the experiment) from the final weight (i.e. weight on final day).

2.2.2.6 Stable isotope analysis

Use of stable isotopes is now a well-accepted method to evaluate the trophic structure and dynamics of ecological communities (Peterson and Fry, 1987; Crawford *et al.*, 2008; Middelburg, 2014; Perkins *et al.*, 2014) and it can be used to test hypotheses in invasion ecology (McCue *et al.*, 2019). Inferences about the diet composition of an animal can be made by analysing stable isotope compositions of a consumer and its food items (Phillips *et al.*, 2014). Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic signatures can represent an organism's trophic niche and thus used in this study to determine underlying mechanisms of signal crayfish invasion at different densities in invaded ecosystem. $\delta^{13}C$ signifies the ‰ difference in the ^{13}C:^{12}C ratio between a sample and a carbonate standard, whereas, $\delta^{15}N$ signifies the ‰ difference in the ^{15}N:^{14}N ratio between a sample and nitrogen (N_2) in air (McCutchan and Lewis, 2001).

Two types of tissue samples were considered for the stable isotope ($\delta^{15}N$ and $\delta^{13}C$) analysis of crayfish and bullhead, (i) muscle tissue from abdominal somites (for signal crayfish) and flank of the tail (for bullhead) and (ii) hepatopancreas (for crayfish) and liver (for bullhead), due to their relatively fast turnover rate (Tieszen *et al.*, 1983; Bondar *et al.*, 2005; Jackson *et al.*, 2014). Muscle, liver and hepatopancreas samples were collected through dissections, after thawing of frozen samples. These were dried at 60°C in an oven for 24 h for muscle, and 48 h for liver / hepatopancreas, to a constant weight. Later these dried samples were pulverised using a mortar and pestle. Finally, ground samples of crayfish muscle (mean ± SD: 0.52 ± 0.09 mg) and hepatopancreas (0.59 ± 0.13 mg) and bullhead muscle (0.54 ± 0.8 mg) and liver (0.59 ± 0.12 mg), were placed in tin capsules for analysis in a mass spectrometer in the Stable

Isotope Biogeochemistry Laboratory (SIBL) of the Department of Earth Sciences, University of Durham. Tissue extraction and sample preparation were carried out by S. Galib, while stable isotope measurements and calibrations were carried out by Dr. D. Grocke who manages Durham's $\delta^{15}N$ and $\delta^{13}C$ stable isotope facility. Precautions were taken to avoid cross-contamination of samples. Common utensils, used in the preparation of samples (e.g. mortar and pestle, tweezers and scoopula), were cleaned using methanol and dried by air between samples.

Sample sizes considered during stable isotope analyses were as follows: 45 muscle and 45 hepatopancreas samples of signal crayfish (one per tissue per individual; three individuals from each enclosure = 15 from each treatment group $T_1 – T_3$); 60 muscle and 60 liver samples of bullhead (one per tissue per individual; three from each enclosure = 15 from each group C_2 and $T_1 – T_3$). Sex ratio of signal crayfish was maintained (~ 1 : 1 ratio; 22 male and 23 female) during selection of crayfish for stable isotope analyses to minimise effects of sexes on outcomes, if any. Three bullhead (i.e. 3 muscle and 3 liver samples) and five signal crayfish (i.e. 5 muscle and 5 hepatopancreas samples), collected from the River Lune (from outside of the enclosures or wild) were also analysed for stable isotope signatures.

Bullhead are principally carnivorous, specialising on benthic invertebrates (Dahl, 1998), whereas signal crayfish are more omnivorous (Stenroth and Nystrom, 2003). Individual benthic macroinvertebrates belonging to different families which are potential prey items for bullhead and signal crayfish and represent different trophic levels in the food web were collected from the experimental site at the end of the enclosure experiment and subjected to stable isotope analyses. For this purpose, samples of Chironomidae (n = 5; five individuals in each), Baetidae (n = 5; five individuals in each), Gammaridae (n = 5; five individuals in each), Heptageniidae (n = 5; three individuals in each), Rhyacophilidae (n = 5; single individual in each) and Hydropsychidae (n = 5; single individual in each) were dried at 60°C in an oven for 24 hours to a constant weight. Differences in numbers of individuals within a sample were due to individual size. Samples were then ground using a mortar and pestle. For

stable isotope analysis of invertebrate prey, 0.59 ± 0.08 mg (mean and SD) dried mass for each sample was used.

The C:N ratios of animal tissue samples (mean ± SD: crayfish muscle, 3.94 ± 0.09; bullhead muscle, 4.10 ± 0.17; crayfish hepatopancreas, 15.53 ± 4.98; bullhead liver, 14.64 ± 4.54; and macroinvertebrates, 6.87 ± 2.38) were greater than 3.5 and this indicates that the amount of lipid present in tissues may negatively affect $\delta^{13}C$ values, but not $\delta^{15}N$ values (Logan and Lutcavage, 2008; Logan *et al.*, 2008; Skinner *et al.*, 2016). Thus, tissue-specific lipid correction models were applied to correct carbon isotope data before analysis. For this purpose the following mathematical models were used: for muscle, $\delta^{13}C_{lipid-free} = \delta^{13}C_{bulk} - 5.16 + 4.527\ ln$ (C:N ratio); for liver or hepatopancreas, $\delta^{13}C_{lipid-free)} = \delta^{13}C_{bulk} - 1.56 + 2.427\ ln$ (C:N ratio); and for invertebrates, $\delta^{13}C_{lipid-free)} = \delta^{13}C_{bulk} - 2.056 + 1.907\ ln$ (C:N ratio) (following Logan *et al.*, 2008).

Although lipid extraction of tissues with high lipid content before stable isotope analysis is quite common, it is not strictly necessary as it can be done accurately with lipid correction equations (Skinner *et al.*, 2016) and is a common practice in recent ecological studies (Le Croizier *et al.*, 2016; Collier *et al.*, 2018; Barton *et al.*, 2019). Therefore mathematical equations were used to correct stable isotope data of different animal tissues in this study.

Because crayfish are omnivorous, stable isotope analyses of several plant materials were also included for dietary assessment. In-stream macrophytes were rare at the study site (restricted to patches of river moss *Fontinalis* sp.), as is typical in many upland Pennine streams where epilithic algae are the main primary producers. Benthic algae, as attached visible algal materials to rocks, were scraped off with a soft toothbrush and deionised water in clean trays at the field site at the end of the enclosure experiment. The samples were then cleaned carefully to remove any foreign particles present in the sample, if any. For stable isotope analysis samples were centrifuged with deionised water and oven-dried prior to homogenisation with a mortar and pestle (Bondar *et al.*,

2005). Five samples (*n* = 5), weighing 0.66 ± 0.12 mg, were used for stable isotope analysis. Samples of in-stream leaf litter, fallen riparian tree leaves (common alder *Alnus glutinosa*), fallen oak *Quercus robur* leaves used in the enclosure (see section 2.2.2.8 leaf litter decomposition) and in-stream woody debris were also collected. These samples were dried for 72 h in an oven at 60°C followed by homogenisation with a mortar and pestle. Five samples (*n* = 5; mean [± SD] sample weight of 1.75 ± 0.18 mg) from each group were analysed for stable isotopes.

No terrestrial invertebrates were considered for stable isotope analysis as potential diet in this this study because they did not appear in Surber samplings (see section 2.2.2.4) for macroinvertebrates. Moreover, both signal crayfish and bullhead tend to feed on benthic prey rather than floating or drifting ones (Western, 1969; Dahl, 1998). In addition, due to the small mesh size used in this study there was limited opportunity for study animals to feed on terrestrial invertebrates.

Ten per cent of the total samples were analysed in duplicates to determine the precision of measurements. Carbon and nitrogen stable isotope analysis of the samples were performed using a Costech Elemental Analyser (model ECS 4010) connected to a Thermo Scientific Delta V Advantage isotope ratio mass spectrometer. Carbon isotope ratios were corrected for ^{17}O and reported in standard delta (δ) notation in per mil (‰) relative to Vienna Pee Dee Belemnite (VPDB) standard. Nitrogen isotope ratios were reported against atmospheric nitrogen (AIR). Isotopic accuracy was monitored through routine analyses of in-house standards, which were stringently calibrated against international standards (e.g., IAEA-600, IAEA-CH-3, IAEA-CH-6, IAEA-N-1, IAEA-N-2, NBS 19, USGS24 and USGS40). International and in-house standards were run daily and provided a linear range for $\delta^{13}C$ between –46‰ and +3‰ and in $\delta^{15}N$ between –4.5‰ and +20.4‰. Analytical uncertainty in carbon and nitrogen isotope analysis was typically ±0.1‰ for replicate analyses of the international standards and <0.2‰ on replicate sample analysis. Total organic carbon and nitrogen data was obtained as part of the isotopic analysis using the internal standard, glutamic acid (40.82 wt% C, 9.52 wt% N).

2.2.2.7 Determination of algal growth

At the time of enclosure deployment, a clean 10 cm × 10 cm unglazed ceramic tile was added into each enclosure to quantify epilithic (periphyton) algal standing stock. These tiles were placed at approximately a 30° angle against the downstream end enclosure wall and they were well exposed to water flow and study animals (i.e. bullhead and signal crayfish). At the end of the experiment, algal colonisation tiles were carefully removed from the enclosures. Algal biofilms were collected into darkened plastic (30 ml) bottles by scrubbing and washing the tiles with a clean toothbrush and deionised water. These were immediately stored at low temperature in a portable cooler box, transported to the laboratory and preserved in a –20°C freezer.

Algal samples were analysed in terms of their chlorophyll-*α* content as an index of algal biomass. Chlorophyll-*α* concentration from the biofilm samples was determined spectrophotometrically (as mg ml^{-1}; following Jeffrey and Humphrey, 1975). Each biofilm sample was thawed and filtered on a 47 mm glass fibre filter paper (GF/C Whatman) and added to a 10 ml solution of 90% acetone. The samples were then stored at 5°C for 24 hours in a lightless spark-free refrigerator for chlorophyll-*α* extraction to occur. The solution was then centrifuged at 2530 rpm for five minutes followed by pouring of subsamples into 5 ml cuvettes. The absorbance was measured at 630, 647 and 664 nm in a spectrophotometer (model GENESYS™ 10S UV-Vis, Thermo Scientific, USA), calibrated with a 90% acetone solution. All the laboratory activities were carried out at the Department of Biosciences and Department of Chemistry of the University of Durham.

2.2.2.8 Determination of rate of leaf litter decomposition

In addition to unglazed tiles for measuring algal standing stock, a mesh pack of 10 mm aperture, filled with 3.00 g of dried oak *Quercus robur* leaf-litter, was also added to each enclosure to measure breakdown rates (after Woodward *et al.*, 2008). The mesh packs were allowed to condition in the enclosures (one per enclosure) for two weeks prior to the start of the experiment (Bondar *et al.*, 2005). On the final day of experiment, leaf litter was removed from each mesh pack and placed immediately into

individually labelled zip-lock bags. Later in the laboratory, macroinvertebrates were separated from the leaf litter samples and these were dried to a constant mass at 60°C in a conventional drying over and then weighed. Breakdown rate was calculated as percentage dry mass loss per day (61 days in total including 14 days of conditioning and 47 days of final experiment). It was assumed that the loss of leaf litter before introducing study organisms (i.e. signal crayfish and bullhead) was very little and similar across enclosures.

2.2.2.9 Water quality parameters

During the pilot experiment, water flow velocity was measured, both outside and inside the enclosures, to determine the effects of the enclosure mesh on water flow inside the enclosure. Flow velocity was measured with an electromagnetic flow meter (Valeport 801, UK) through a series of transects at 60% depth of water (25%, 50% and 75% width positions within enclosures, across transects at the downstream end, middle and upstream end of each enclosure; n = 9 at each enclosure). A similar number of measurements were also recorded between 0.5 and 2 m upstream of the enclosures. The effect of mesh on water flow was small, usually a <10% reduction inside of the enclosures.

During the enclosure experiment, an automatic water depth and temperature recording logger (model: Hobo water level logger, Onset Computer Corporation, MA, USA) was set before starting the experiment to record both parameters every 15 minutes until the end of the field study. Water depth, water temperature, pH, dissolved oxygen (DO) and flow velocity within each enclosure were also recorded weekly, between 1000 and 1200 hours on each sampling day.

2.2.3 Statistical tests

All the analyses were performed in statistical software R (version 3.4.3; R Core Team, 2017), with an α level of significance of 0.05. Before analysis, data were explored following Zuur *et al.* (2010) to avoid common statistical problems.

Linear Mixed-Effects Modelling (LMM) was employed to analyse repeated measures macroinvertebrate richness and abundance data using the 'lmer' function of the 'lme4' package (Bates *et al.*, 2015) and *P*-values were obtained from the 'lmerTest' package (Kuznetsova *et al.*, 2016). During analysis, experimental groups (five levels, $C_1 – C_2$ and $T_1 – T_3$), time (two levels, before and after) and their interaction (group × time) were considered fixed effects and replications (i.e. enclosure ID), nested within experimental blocks, were considered a random effect. To determine the dissimilarities among macroinvertebrate communities across groups, time, and their interaction, a Permutational Multivariate Analysis of Variance (PERMANOVA), using distance matrices and 999 permutations, was carried out by employing the 'adonis2' function of the 'vegan' package (Oksanen *et al.*, 2018). During PERMANOVA analysis factors were considered nested within 'blocks'.

Similarity Percentage Analysis (SIMPER), based on decomposition of Bray-Curtis dissimilarity index (Clarke, 1993) and 999 permutations, was used to determine the average percent dissimilarity over time (before vs. after) and space (control and treatment groups) and to identify the contribution of individual macroinvertebrate families, belonging to each experimental group, responsible for average dissimilarity between 'before' and 'after' communities. Macroinvertebrate families that accounted for the differences between before and after communities were identified from SIMPER analyses based on the ratio between the average contribution to dissimilarity and the standard deviation which is a measure of the how consistently a species or family contributes to dissimilarity over time (Solomon *et al.*, 2016).

Body weight gain of bullhead between control and treatment groups was also compared using LMM as outlined above. During analysis experimental groups (control and treatment) were considered fixed effect and replications were considered random effects. A nested Analysis of Variance (ANOVA) was used to analyse data of algal standing stock and leaf-litter breakdown to determine the impacts of signal crayfish density by comparing control and treatment groups. Nested ANOVA was performed by defining 'experimental block' as a random effect in the model using the

R packages mentioned earlier. Post-hoc comparisons of the mean values of control and treatments groups were obtained by using the 'glht' function of the 'multcomp' package (Hothorn *et al.*, 2008).

To allow comparison between groups, the standardised effect size, Hedges' *g* (Hedges, 1981), was also calculated by comparing crayfish treatment groups (i.e. $T_1 – T_3$) with the control groups, C_1 (no crayfish or bullhead) and C_2 (bullhead only). The R package 'effsize' (Torchiano, 2018) was used for the calculation of effect size.

Repeated measures physico-chemical properties of water (water temperature, water depth, water flow, DO, and pH) recorded from enclosures were analysed using LMM outlined earlier with groups (i.e. controls and treatments) as fixed effect and replications (nested within blocks) as random effect.

Before analysis, data were checked for normality by Shapiro–Wilk test (Peat and Barton, 2005) and necessary transformations (square-root transformation for macroinvertebrate abundance data, McDonald, 2014; and log (x + 1) transformation for water quality data, Clarke, 1993) were made to meet the assumptions for the test.

To analyse stable isotope data, Stable Isotope Mixing Models (SIMMs) were used using the "simmr" package (Parnell *et al.*, 2010, 2013) in R (R Core Team, 2017). Using SIMMs, with the default priors, diets for both signal crayfish and bullhead were quantified. To better represent the outcomes, crayfish and bullhead data were analysed separately. In order to correct data, Diet-Tissue Discrimination Factor (DTDF) values were added to the food source isotope values before SIMM analysis (Phillips *et al.*, 2014) because a consumer's tissue generally has higher isotopic values for nitrogen and carbon than its prey items (i.e. diet) due to discrimination during assimilation and excretion processes (Olive *et al.*, 2003). It is important to use accurate DTDF when estimating the assimilated diets of free-ranging animals (Wolf *et al.*, 2009). For signal crayfish, DTDF values of +2.0‰ and +2.3‰ were used for carbon and nitrogen respectively (Rudnick and Resh, 2005; Wood *et al.*, 2017). These values were added to different food sources including leaf litter, organic debris, algae and various

families of macroinvertebrates. Although cannibalism in signal crayfish is common (Houghton *et al.*, 2017) crayfish was not included in the model as a potential food source as no signal crayfish including YoY was recorded during invertebrate sampling collected through Surber sampler and there were no missing crayfish in any of the enclosures. For bullhead, a DTDF value of +2.1‰ was used for carbon (McCutchan *et al.*, 2003). A DTDF value of +2.3‰ for the nitrogen isotope ($\delta^{15}N$) was obtained through the calculation of the mean DTDF value from those reported in fishes feeding on prey items similar to those of bullhead (i.e. primarily macroinvertebrates). These fishes were *Coregonus nasus* (+2.0‰; Hesslein *et al.*, 1993), *Oncorhynchus mykiss* (+1.3‰ and +1.9‰; Rounick and Hicks, 1985; McCutchan *et al.*, 2003) and *Salvelinus fontinalis* (+3.3‰; McCutchan *et al.*, 2003). Potential food sources for bullhead would be different families of macroinvertebrates, bullhead eggs, newly-hatched bullhead and signal crayfish (Western, 1969; Marconato and Bisazza, 1988; Coop *et al.*, 1994; Dahl, 1998). However, only different families of macroinvertebrates were considered during modelling as no smaller signal crayfish and bullhead eggs or larvae were recorded from any of the enclosures during macroinvertebrate sampling.

Nitrogen and carbon isotopic values of different signal crayfish and bullhead groups were compared using the LMMs in which groups (five levels; wild, C_2, T_1–T_3) were tested as fixed effect and crayfish sources (i.e. enclosure IDs and wild) as a random effect. As two isotopes ($\delta^{15}N$ and $\delta^{13}C$) were considered in this study, only up to three prey sources (n + 1, where n is the number of isotope analysed) can be used in SIMM to calculate a unique solution for prey sources (Phillips and Gregg, 2003; Inger *et al.*, 2006). Therefore an *a priori* aggregation approach was used in this case whereby source data (i.e. isotopic values) were plotted and similar sources forming clusters were grouped before analysis (Phillips *et al.*, 2005, 2014).

During modelling of bullhead diet, macroinvertebrate families with no statistically significant difference were assigned to three groups based on their ecological roles and $\delta^{13}C$ values after examining pairwise comparisons of macroinvertebrate families, obtained through one way

ANOVA followed by a post–hoc (Tukey HSD) test (Ben-David, Flynn, *et al.*, 1997; Ben-David, Hanley, *et al.*, 1997; Phillips *et al.*, 2005). These groups were as follows: chironomids (Chironomidae), grazers and shredders (Gammaridae, Hydropsychidae, Heptageniidae and Baetidae), predatory caddis (Rhyacophilidae). For modelling of signal crayfish diet, leaf litter (with debris) and algae were also considered due to the omnivorous feeding nature. For crayfish, in order to reduce the number of potential prey groups to three (see above) all macroinvertebrates were treated as a single group (Phillips *et al.*, 2005; Fry, 2013; Petitet and Bugoni, 2017). Only muscle sample data were considered for SIMMs as tissues with high lipid content (i.e. liver for bullhead or hepatopancreas for signal crayfish in this study) can negatively affect carbon and nitrogen isotopic signature values by preventing them reaching isotopic equilibrium (Chen *et al.*, 2012). Muscle tissue is considered the most consistent proxy for stable isotopes in trophic studies, by comparison to other tissues (Hesslein *et al.*, 1993; Pinnegar and Polunin, 1999; Chen *et al.*, 2012). Dietary changes, indicated by $\delta^{15}N$ and $\delta^{13}C$ in this study, occurred similarly in muscle to shorter-turnover tissues such as liver, rendering muscle stable isotope data valid for SIMM analyses (see section 2.3.3).

2.3 Results

2.3.1 Macroinvertebrates

2.3.1.1 Richness, abundance and community

Before introducing signal crayfish and bullhead to the enclosures, mean (± SD) taxonomic richness of macroinvertebrate was similar across all experimental groups, and varied from 11.8 ± 2.9 (in T_3) to 12.4 ± 3.3 (in T_2) families (Table 2.2). Mean macroinvertebrate abundance was also similar during this time, and ranged between 176.6 ± 54.9 per 0.3 m^2 Surber area (3 samples × 0.1 m^2 Surber; in C_2) and 187.4 ± 54.1 (in T_2). On the final day of experiment, these values varied from 3.8 (T_3) to 12.4 (C_1) families for taxonomic richness and 35.8 (T_3) to 180.4 (C_1) individuals for macroinvertebrate abundance (Table 2.2).

Strongly significant effects of groups, time and their interaction were recorded for macroinvertebrate taxonomic richness (all $P < 0.001$; Table 2.3). Before introducing study animals (bullhead and signal crayfish) the taxonomic richness of macroinvertebrates did not vary significantly among groups ($P = 0.690$) but significant variation was recorded at the end of the study ($P < 0.001$; Table 2.3). There was no significant difference in taxonomic richness between C_1 (without fish or crayfish) and C_2 (bullhead only) ($P = 0.670$) but both these groups differed significantly from treatment groups (T_1–T_3) with signal crayfish except for C_2 vs. T_1 (Table 2.4).

TABLE 2.2 Abundance and taxonomic richness (mean ± SD) in different experimental groups (C_1, without crayfish or bullhead; C_2, bullhead only; T_1, T_2, and T_3 are low, medium and high crayfish density treatments respectively). Abundance is based on combined 3 × 0.1 m^2 Surbers per enclosure.

Groups	Taxonomic richness		Abundance	
	Before	After	Before	After
C_1 (Ctrl)	12.1 ± 2.8	12.4 ± 2.8	179.5 ± 50.8	180.4 ± 61.2
C_2 (Bull)	12.0 ± 2.5	11.0 ± 2.8	176.6 ± 54.9	150.6 ± 49.0
T_1 (Low)	12.2 ± 3.3	9.2 ± 1.8	180.6 ± 60.3	85.4 ± 49.7
T_2 (Medium)	12.4 ± 3.3	7.8 ± 2.3	187.4 ± 54.1	64.2 ± 45.3
T_3 (High)	11.8 ± 2.9	3.8 ± 0.8	180.6 ± 55.1	35.8 ± 23.0

For macroinvertebrate abundance, despite no significant variation between control and treatment groups before bullhead and signal crayfish introduction to the enclosures (based on combined Surber replicates, $n = 5$ per treatment; LMM, $P > 0.05$) they varied strongly at the end of the study (LMM, $P < 0.001$; Table 2.3, Figure 2.3). Strong effects of group, time and their interaction were recorded (all $P < 0.001$; Table 2.3). Post-hoc testing confirmed that there was no significant variation in macroinvertebrate abundance between the two control groups (i.e. C_1 vs. C_2; $P = 0.112$) but both control groups varied highly significantly from the three treatment groups (all $P < 0.001$; Table 2.4).

The macroinvertebrate community did not vary significantly across control and treatment groups before the introduction of bullhead and signal crayfish to the enclosures (PERMANOVA, $P = 0.255$) but they varied significantly at the end of the experiment (PERMANOVA, $P = 0.001$; Table 2.3) which indicates strong impacts of the crayfish and bullhead on the macroinvertebrate community composition. Significant effects of groups, time and their interaction were also recorded for macroinvertebrate communities (all $P \leq 0.01$; Table 2.3).

TABLE 2.3 Macroinvertebrate richness, abundance and community in different control and treatment groups over time. Richness and abundance data were subjected to Linear Mixed-Effects Modelling (LMM) and community data were analysed by Permutational Multivariate Analysis of Variance (PERMANOVA). Pairwise comparisons were made by LMM.

Categories	Comparisons	Mean square	*df*	*F*-value	*P*-value
Richness	Groups	0.090	4, 36	31.4	**<0.001**
	Time	0.326	1, 36	113.5	**<0.001**
	Interaction	0.084	4, 36	29.3	**<0.001**
	Time: Before	0.001	4, 16	0.7	0.690
	Time: After	0.174	4, 16	38.3	**<0.001**
Abundance	Groups	0.614	4, 36	58.1	**<0.001**
	Time	4.364	1, 36	413.1	**<0.001**
	Interaction	0.724	4, 36	68.5	**<0.001**
	Time: Before	0.004	4, 16	2.1	0.130
	Time: After	1.333	4, 16	85.7	**<0.001**
Community	Groups	0.173	4, 40	1.8	**0.005**
	Time	1.005	1, 40	10.6	**0.001**
	Interaction	0.178	4, 40	1.9	**0.003**
	Time: Before	0.005	4, 20	0.06	0.099
	Time: After	0.345	4, 20	3.04	**0.001**

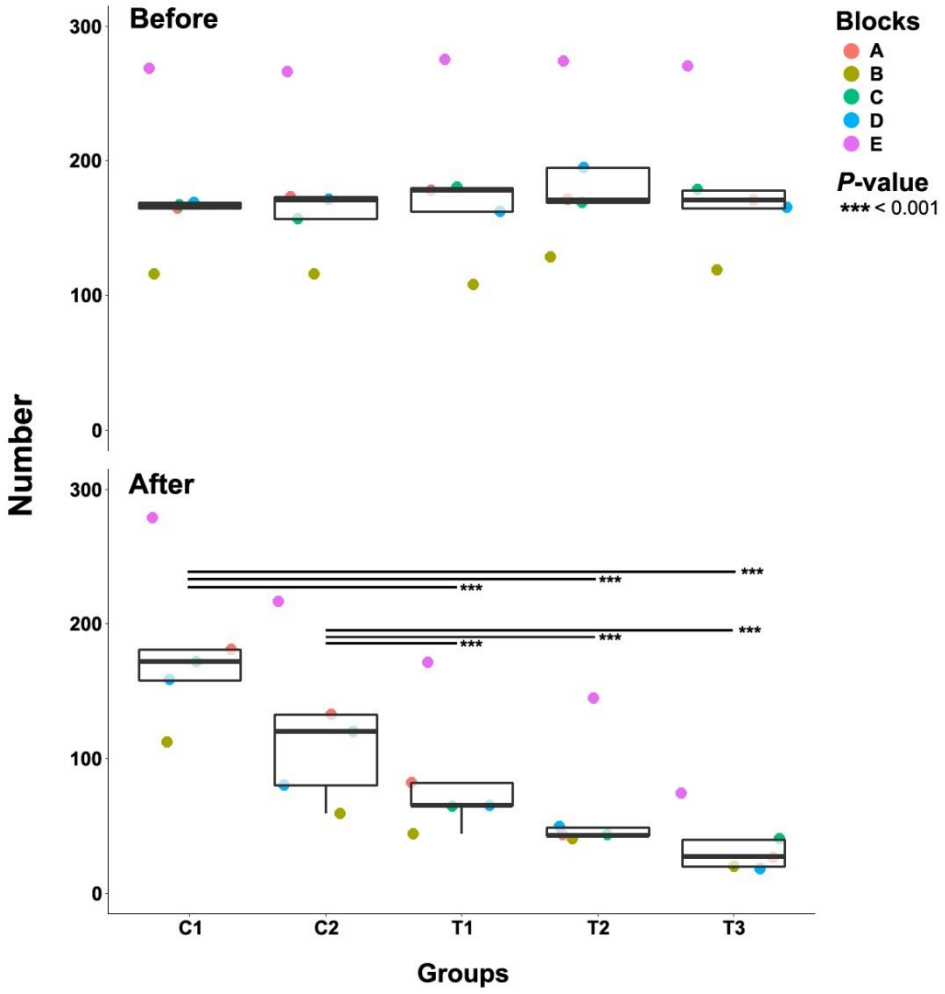


FIGURE 2.3 Abundance of macroinvertebrate (0.3 m^{-2}) in different control and treatment groups (C_1, control without fish or crayfish; C_2, bullhead control; T_1, T_2 and T_3 are low, medium and high crayfish density treatments respectively) over time. Midline within the box is the median; upper and lower limits of the box represent the third and first quartile (75th and 25th percentile) respectively. Points are individual enclosure data.

TABLE 2.4 Pairwise comparisons of macroinvertebrate abundance within and between control and treatment groups (C_1, control without fish or crayfish; C_2, bullhead control; T_1, T_2 and T_3 are low, medium and high crayfish density treatments respectively).

Comparisons	Richness		Abundance	
	***z*-value**	***P*-value**	***z*-value**	***P*-value**
C_2–C_1	–1.3	0.670	–2.4	0.112
T_1–C_1	–3.1	**0.016**	–10.1	**<0.001**
T_2–C_1	–4.9	**<0.001**	–13.5	**<0.001**
T_3–C_1	–11.2	**<0.001**	–19.2	**<0.001**
T_1–C_2	–1.8	0.393	–7.7	**<0.001**
T_2–C_2	–3.6	**0.003**	–11.1	**<0.001**
T_3–C_2	–9.9	**<0.001**	–16.8	**<0.001**
T_2–T_1	–1.8	0.378	–3.4	**0.006**
T_3–T_1	–8.1	**<0.001**	–9.1	**<0.001**
T_3–T_2	–6.3	**<0.001**	–5.7	**<0.001**

2.3.1.2 Changes in macroinvertebrate families

At the end of the study a dramatic decrease in the abundance of macroinvertebrate families was recorded in treatment groups with signal crayfish (Table 2.5). SIMPER results showed that no macroinvertebrate families differed significantly before and after situations in control group without crayfish and bullhead (i.e. C_1; Tables 2.5 & S2.2). In the control group with bullhead (C_2), only Hydropsychidae decreased significantly over time ($P = 0.033$; Tables 2.5 & S2.3). Whereas, in T_1, the abundance of six macroinvertebrate families (Gammaridae, Elmidae, Dixidae, Leuctridae, Culicidae and Hydrophilidae; all $P \leq 0.018$) decreased significantly over time (Tables 2.5 & S2.4). In the medium density treatment group (T_2) the abundance of eight macroinvertebrate families decreased significantly over time (all $P \leq 0.023$; Tables 2.5 & S2.5). This number was even higher for the high density treatment group (T_3) where a significant decrease in abundance was recorded for 13 macroinvertebrate families (all $P \leq 0.022$; Tables 2.5 & S2.6).

TABLE 2.5 Changes in different families belonging to various groups and their contribution to overall dissimilarities in communities over time (before vs. after), obtained through Similarity Percentage Analysis (SIMPER) analysis. Arrow direction indicates decrease, stable or increase.

Families	Changes (contribution to dissimilarities) (%)				
	C_1	C_2	T_1	T_2	T_3
Heptageniidae	↑1 (10.6)	↓6.6 (10.6)	↓31.7 (15.3)	↓39.9 (15.8)	↓51.1 (15.5)
Chironomidae	↑17 (10.6)	↑10.4 (11.6)	↓1.5 (9)	↓19.2 (7.2)	↓25.5 (7.1)
Simulidae	↑22.3 (8.8)	↓0.3 (8.6)	↓36.9 (7.3)	↓56.9 (7)	↓86.2 (6)
Baetidae	↑0.8 (8.1)	↑2.9 (8.2)	↓30.5 (7)	↓44.2 (7.3)	↓62.7 (7.4)
Dixidae	↓0.2 (7.8)	↓42.3 (6.4)	↓100* (4.5)	↓100** (4)	↓100** (3.9)
Nemouridae	↓10.5 (6.3)	↓18.1 (7.1)	↓14.9 (5.2)	↓30.7 (4.7)	↓100** (4.8)
Ephemerellidae	↑9.8 (6.1)	↓28.9 (6)	↓50.2 (5.9)	↓73.2 (6.2)	↓85 (5.5)
Caenidae	↓6.2 (5.3)	↓22.1 (5.3)	↓49.3 (4.7)	↓79.3 (4.5)	↓100** (4)
Leptophebiidae	15.5 (4.3)	↓10.6 (3.6)	↓49.8 (3.5)	↓56.3 (2.9)	↓100** (2.5)
Rhyacophilidae	↓8.8 (4.3)	↓100 (3.8)	↓77.3 (3.2)	↓100** (3)	↓100** (2.9)
Perlodidae	↓1.6 (3.9)	↓4.2 (3.4)	↓75 (4.1)	↓86.3* (4.9)	↓100** (4.1)
Leuctridae	↓1.2 (3.8)	↓7.8 (4)	↓31.5* (4.4)	↓58.7* (6.7)	↓52.8 (6.5)
Culicidae	↓10.1 (3.3)	↓17.1 (2.6)	↓100* (2)	↔0 (1.7)	↓100** (1.7)
Tipulidae	↓18.4 (3.1)	↓18.4 (3.2)	↓50 (2.6)	↓100** (1.7)	↓100** (1.4)
Hydropsychidae	↓14.2 (2.9)	↓42.2* (5.3)	↓48.9 (6)	↓63 (6.2)	↓100** (9.4)
Elmidae	↓5.9 (2.9)	↓31 (4.4)	↓68.3* (5.1)	↓87.7* (5.5)	↓87.6* (4.8)
Gammaridae	↑14 (2.4)	↓4.5 (3.3)	↓51.9* (7.2)	↓71.7** (8.9)	↓94.3** (11)
Perlidae	↑22.5 (2.3)	↓100 (1.3)	↓42.3 (1.8)	↓100** (1)	↓100** (1.1)
Hydrophilidae	↑22.5 (2.3)	↔0 (1.5)	↓100* (1.3)	↓100** (0.9)	↓100** (0.9)
Polycentropodidae	↑100 (0.9)	–	-	-	-

↑, increased; ↓, decreased; ↔, unchanged; *, $P < 0.05$; **, $P < 0.01$

Comparing macroinvertebrate community change by abundance of families at the end of study, these were quite similar in both control groups (C_1 and C_2) and no significant difference was recorded in the relative abundance of any of the families between two groups (Table S2.7). However, nearly significant (P = 0.077) decrease was recorded for predatory caddisfly family Rhyacophilidae. In T_1, when compared to C_1, the abundance of six families declined significantly (Table S2.8) whereas this figure was 8 and 15 families for T_2 and T_3 respectively when compared to C_1 (Tables S2.9–S2.10).

Similar trends of macroinvertebrate families were also found for treatment groups containing crayfish (T_1–T_3) when compared to the control group with native benthic fish (C_2, bullhead only). In this case, the abundance of 4, 5 and 11 families decreased significantly in T_1, T_2 and T_3 respectively, relative to C_2 (Tables S2.11–S2.13).

2.3.2 Growth of bullhead

Despite no significant variation in initial body weight of bullhead across groups (LMM; F = 0.74, P = 0.529) they varied significantly at the end of the study (F = 3.86, P = 0.012). A strong impact on bullhead growth, in terms of weight gain, was recorded, in which reduced growth rates were recorded for bullhead from treatment groups when compared to controls (LMM, F = 33.25, P < 0.001; Table 2.6, Figure 2.4). Post-hoc tests confirmed that the weight gained by bullhead was negatively related to the density of signal crayfish. Bullhead in T_3 actually lost weight (negative growth) by 0.18 ± 0.36 g. Bullhead weight gain in T_1 (0.21 ± 0.1 g) and T_2 (0.05 ± 0.1 g) was lower than bullhead from control group (C_2; 0.38 ± 0.15 g; Figure 2.4). At the end, two bullhead were missing from two enclosures belonging to the high-density treatment group, T_3.

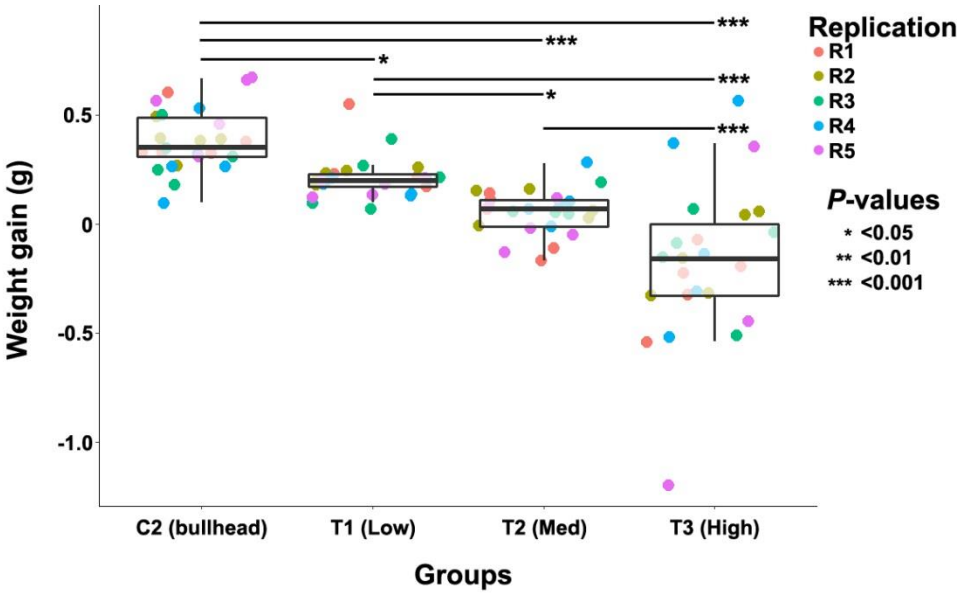


FIGURE 2.4 Body weight gain (g) of bullhead belonging to different groups (C_2, bullhead control; T_1, T_2 and T_3 are low, medium and high crayfish density treatments respectively). Midline within the box is the median; upper and lower limits of the box represent the third and first quartile (75th and 25th percentile) respectively. Points are individual data.

TABLE 2.6 Weight gain of bullhead in control and treatment groups (C_2, bullhead control; T_1, T_2 and T_3 are low, medium and high crayfish density treatments respectively), obtained through Linear Mixed-Effects Modelling (LMM).

Groups	Group types	*z*-value	*P*-value
C_2 vs. T_1	Control (bullhead) – Low SC	3.05	0.012
C_2 vs. T_2	Control (bullhead) – Medium SC	5.73	<0.001
C_2 vs. T_3	Control (bullhead) – High SC	9.61	<0.001
T_1 vs. T_2	Low SC – Medium SC	2.68	0.038
T_1 vs. T_3	Low SC – High SC	6.62	<0.001
T_2 vs. T_3	Medium SC – High SC	4.04	<0.001

2.3.3 Stable isotope analysis

A clear separation between the isotopic niche spaces of bullhead and signal crayfish was evident from the stable isotope analyses of both muscle and liver or hepatopancreas tissues (Figures 2.5 & 2.6). Bullhead occupied a higher trophic position (measured as $\delta^{15}N$) than signal crayfish in the food web, apparent both from muscle (mean ± SD: 7.11 ± 0.46‰ for signal crayfish and 8.93 ± 0.27‰ for bullhead) and hepatopancreas or liver tissues (3.89 ± 0.62‰ for signal crayfish and 7.76 ± 0.4‰ for bullhead) (Figures 2.5 & 2.6). Several outlier values for crayfish were checked with duplicated samples and were consistent, suggesting they were genuine.

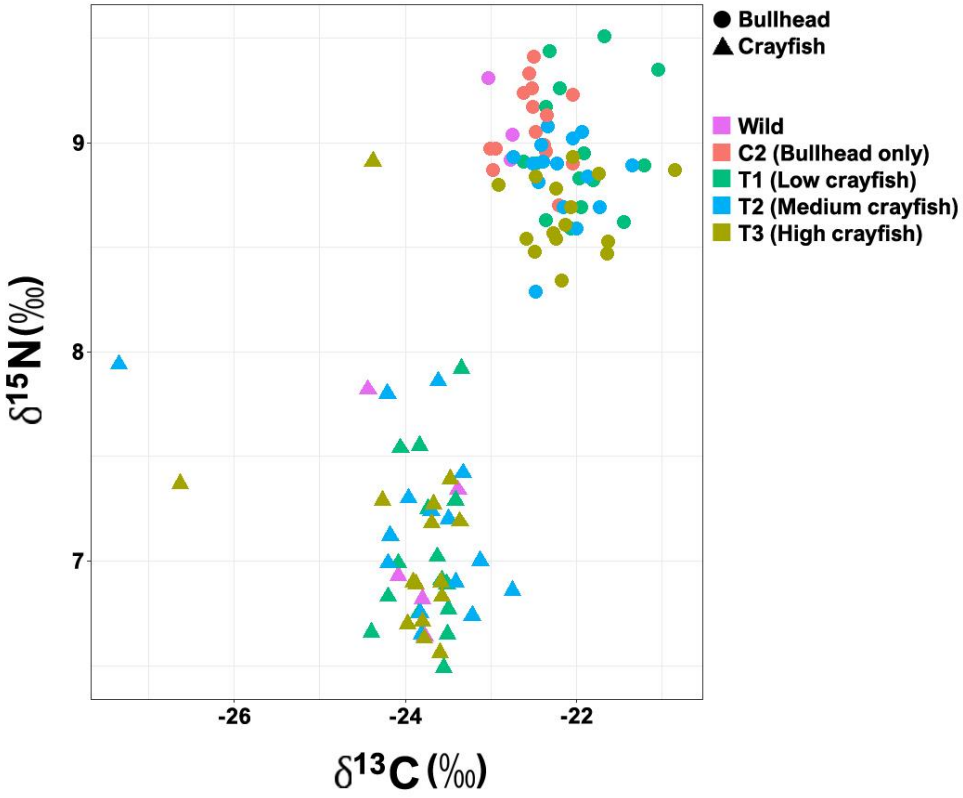


FIGURE 2.5 Nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotopic signatures in muscle of signal crayfish and bullhead after 47 days in experimental enclosures in the River Lune, based on stable isotope analyses.

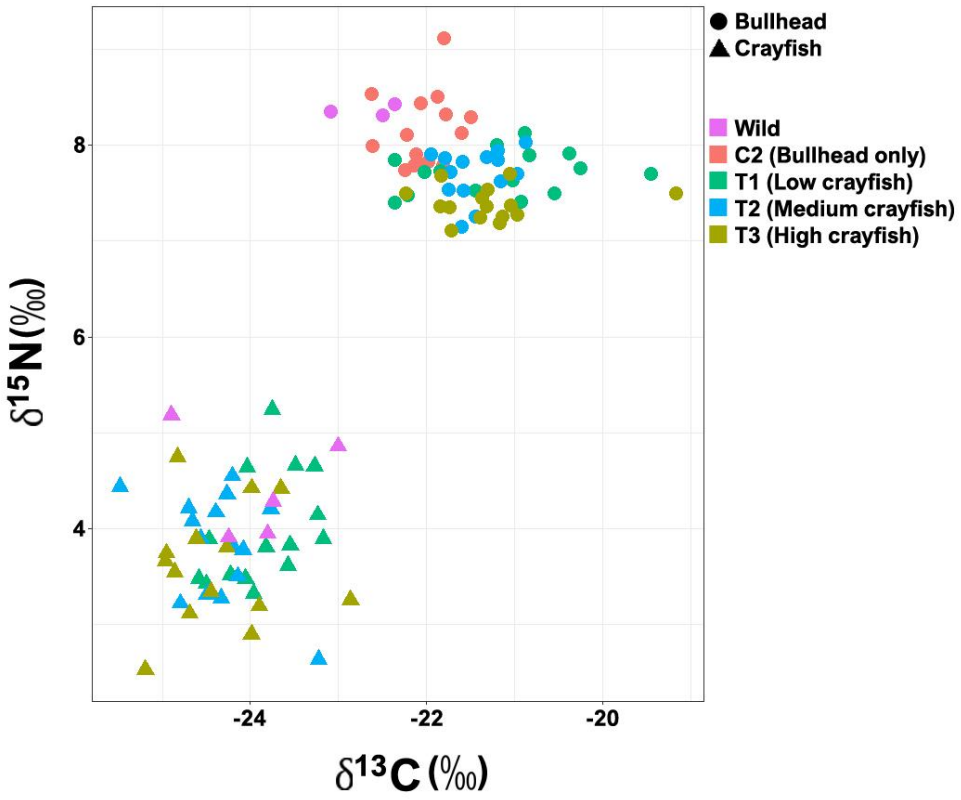


FIGURE 2.6 Nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotopic signatures in signal crayfish hepatopancreas and bullhead liver after 47 days in experimental enclosures in the River Lune, based on stable isotope analyses.

Mean (± SD) values of carbon isotope ($\delta^{13}C$) in muscle samples were –23.87 ± 0.74‰ for signal crayfish and –22.23 ± 0.48‰ for bullhead. These values were –24.16 ± 0.58‰ for crayfish hepatopancreas and –21.54 ± 0.7‰ for bullhead liver tissues. For signal crayfish, $\delta^{13}C$ was significantly lower in hepatopancreas (paired *t*-test: $P = 0.03$). $\delta^{15}N$ value was also significantly lower in crayfish hepatopancreas than muscle (paired *t*-test: $P < 0.001$). Significantly lower isotopic values of $\delta^{15}N$ and $\delta^{13}C$ were also recorded in liver of bullhead than muscle tissue (both $P < 0.001$). However, there was no significant differences in carbon and nitrogen isotopic values between male and female signal crayfish for both muscle (*t*-tests: $t = 0.44$, $P = 0.661$ for $\delta^{13}C$; $t = –0.47$, $P = 0.643$ for $\delta^{15}N$) and hepatopancreas (*t*-tests: $t = –0.87$, $P = 0.392$ for $\delta^{13}C$; $t = 0.29$, $P = 0.775$ for $\delta^{15}N$) tissues.

Although isotope values were significantly lower in hepatopancreas tissue of signal crayfish and liver tissue of bullhead than muscle tissues, significant linear relationships were found between tissue types for both species (all $P < 0.001$; Figures 2.7 & 2.8). Isotopic values were positively correlated between tissue types (muscle and hepatopancreas for signal crayfish and muscle and liver for bullhead; for $\delta^{13}C$: $R^2 = 0.39$ [signal crayfish] and 0.58 [bullhead]; for $\delta^{15}N$: $R^2 = 0.42$ [signal crayfish] and 0.40 [bullhead]) (Figures 2.7 & 2.8).

In both muscle and hepatopancreas tissues of signal crayfish, there was no significant variation in the two isotopic signatures (i.e. $\delta^{15}N$ and $\delta^{13}C$) among the three crayfish treatment groups (all $P > 0.05$; Table 2.7). Nonetheless, both $\delta^{15}N$ and $\delta^{13}C$ isotope values in muscle and liver tissues of bullhead differed significantly among groups (both $P < 0.001$; Table 2.7). For $\delta^{15}N$ in bullhead muscle, higher isotopic values were measured in individuals from control enclosures (mean ± SD, 9.12 ± 0.16‰) and wild source (9.12 ± 0.17‰) than individuals from treatment groups with signal crayfish (T_1–T_3; 8.68‰ – 8.97‰) (Figure 2.5). Post-hoc tests revealed that $\delta^{15}N$ values in muscle tissue of T_3 bullhead were significantly lower than other groups (Table 2.8). The medium crayfish density treatment group (i.e. T_2) also had significantly lower $\delta^{15}N$ than the bullhead control group (C_2) (Table 2.8). For $\delta^{13}C$ content in muscle, individuals from both the bullhead control (i.e. C_2) and wild source differed significantly from all treatment groups (T_1–T_3) with signal crayfish (all $P < 0.05$; Table 2.8).

For liver tissue of bullhead, a similar pattern of variation was evident in $\delta^{15}N$ and $\delta^{13}C$ isotope values among groups, as was seen for muscle. $\delta^{15}N$ values were significantly lower in T_3 individuals than other groups (all $P < 0.05$; Table 2.8). $\delta^{15}N$ values were also significantly higher in C_2 than two other treatment groups (i.e. T_1 and T_2). $\delta^{15}N$ values of wild bullhead were also significantly higher than T_1 or T_2 (Table 2.8). For $\delta^{13}C$ values in bullhead liver, all three treatment groups (T_1–T_3) with signal crayfish were with significantly higher values than bullhead control (C_2) and wild source individuals (Table 2.8).

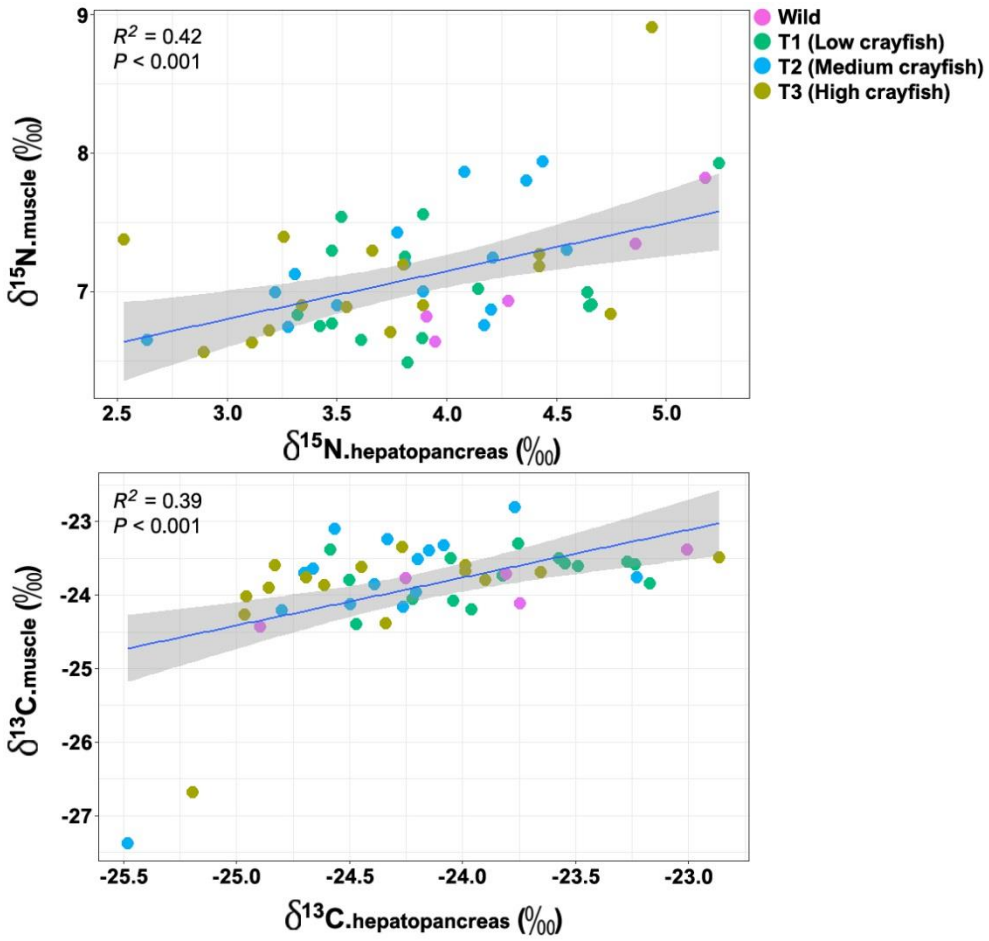


FIGURE 2.7 Relationships between signal crayfish hepatopancreas and muscle tissue isotope values ($\delta^{13}C$ and $\delta^{15}N$) from individuals of various treatment groups. Linear fit with 95% confidence interval represented by grey-shaded areas showing significant trends.

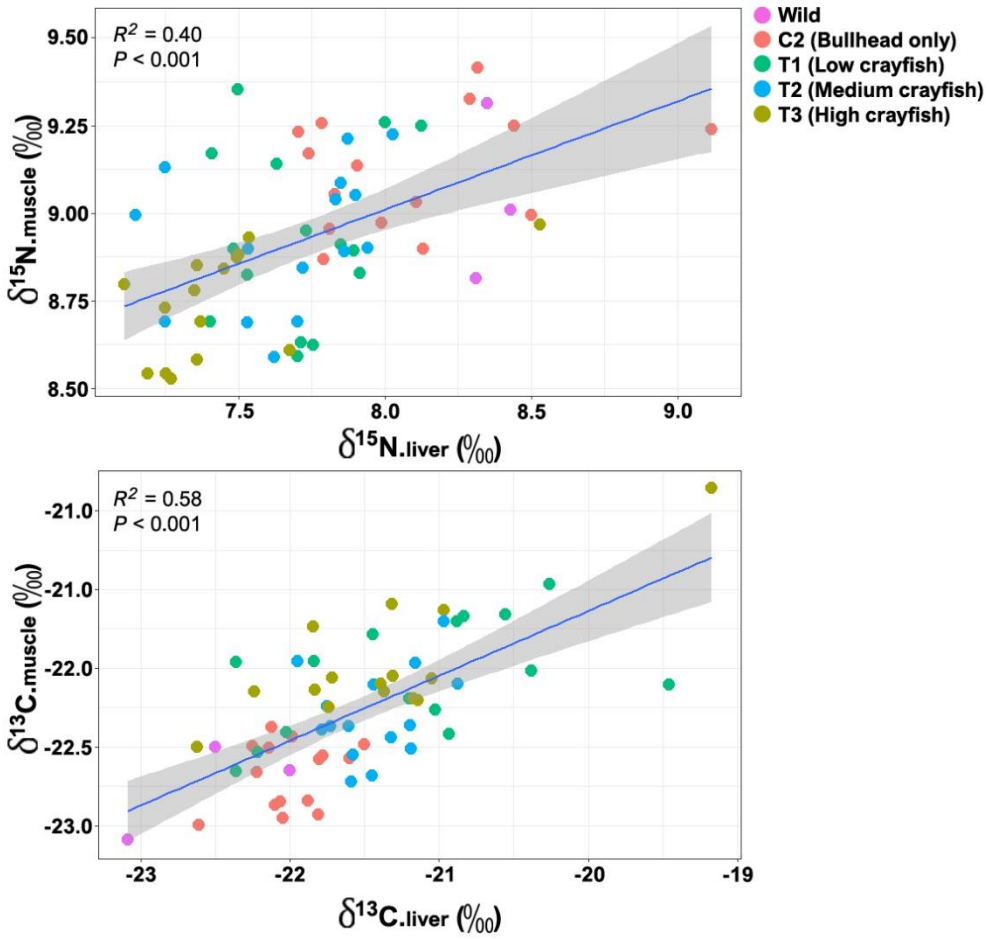


FIGURE 2.8 Relationships between bullhead liver and muscle tissue isotope values ($\delta^{13}C$ and $\delta^{15}N$) from individuals of various treatment groups. Linear fit with 95% confidence interval represented by grey-shaded areas showing significant trends.

TABLE 2.7 Variations in stable isotopic signatures in muscle and liver or hepatopancreas tissues of signal crayfish and bullhead, obtained through Linear Mixed-Effects Modelling (LMMs).

Sources	Tissue	Isotopes	Mean square	*df*	*F* value	*P* value
Signal crayfish	Muscle	$\delta^{15}N$	0.085	2, 42	0.39	0.679
		$\delta^{13}C$	0.162	2, 42	0.29	0.752
	Hepatopancreas	$\delta^{15}N$	0.059	2, 42	0.33	0.724
		$\delta^{13}C$	0.065	2, 42	0.34	0.799
Bullhead	Muscle	$\delta^{15}N$	0.348	3, 56	7.15	**<0.001**
		$\delta^{13}C$	1.267	3, 56	8.75	<**0.001**
	Liver	$\delta^{15}N$	1.147	3, 56	20.16	**<0.001**
		$\delta^{13}C$	1.964	3, 56	6.68	**<0.001**

TABLE 2.8 Pairwise post-hoc comparisons of stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) in muscle among different groups of bullhead (W, wild; C_2, bullhead control; T_1, T_2 and T_3 are low, medium and high crayfish density treatments respectively).

Groups	Muscle				Liver			
	$\delta^{15}N$		$\delta^{13}C$		$\delta^{15}N$		$\delta^{13}C$	
	z	*P*	*z*	*P*	*z*	*P*	*z*	*P*
W vs. C_2	0.06	1.000	–0.61	0.972	0.87	0.904	–1.84	0.341
T_1 vs. C_2	–1.82	0.347	4.96	**<0.001**	–4.31	**<0.001**	4.57	**<0.001**
T_2 vs. C_2	–2.79	**0.039**	3.53	**0.003**	–2.65	**0.047**	2.78	**0.040**
T_3 vs. C_2	–5.38	**<0.001**	4.97	**<0.001**	–7.77	**<0.001**	2.87	**0.031**
T_2 vs. T_1	–0.71	0.952	–1.42	0.600	–0.26	0.999	–1.78	0.371
T_1 vs. T_3	3.54	**0.003**	–0.02	1.000	2.82	**0.036**	1.70	0.421
T_2 vs. T_3	2.83	**0.035**	–1.44	0.589	2.84	**0.034**	–0.08	0.999
W vs. T_1	1.06	0.818	–3.47	**0.005**	3.07	**0.017**	–4.47	**<0.001**
W vs. T_2	1.48	0.563	–2.65	**0.050**	3.19	**0.011**	–3.44	**0.005**
W vs. T_3	3.12	**0.015**	–3.48	**0.004**	4.62	**<0.001**	–3.49	**0.004**

Stable isotope mixing model (SIMM) results for signal crayfish indicated that there were almost no changes in consumption of leaf litter and debris, algae and macroinvertebrates across crayfish treatment groups (Table 2.9, Figure 2.9). Macroinvertebrates was the dominant group in signal crayfish diets with mean contributions of 75.9 – 78.1% of the total diet amount followed by leaf litter and debris (17.4 – 18.9%) and algae (4.4 – 5.2%; Table 2.9, Figure 2.10).

TABLE 2.9 Quantification of signal crayfish diets, based on Stable Isotope Mixing Model (SIMM).

Groups	Crayfish diets (%, mean ± SD; 95% CI)		
	Leaf litter & debris	**Algae**	**Macroinvertebrate**
T_1	18.9±0.05 (7.9–29.5)	5.2±0.03 (1.2–11)	75.9±0.06 (64–88)
T_2	17.4±0.06 (6.5–28.1)	4.5±0.03 (1–10.2)	78.1±0.06 (66.2–90.1)
T_3	18.7±0.06 (7.2–29.3)	4.4±0.02 (0.9–10.1)	76.9±0.06 (65.2–89.2)

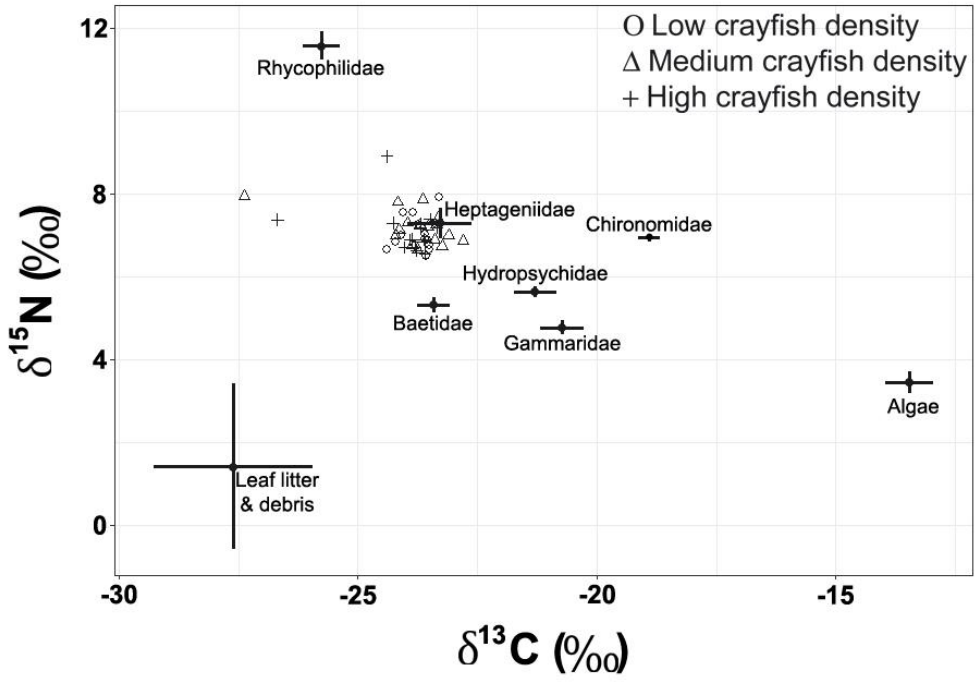


FIGURE 2.9 Isospace plot for three experimental groups of signal crayfish based on stable isotope signatures of carbon and nitrogen and their prey items. Data for prey groups are represented as mean and standard deviation.

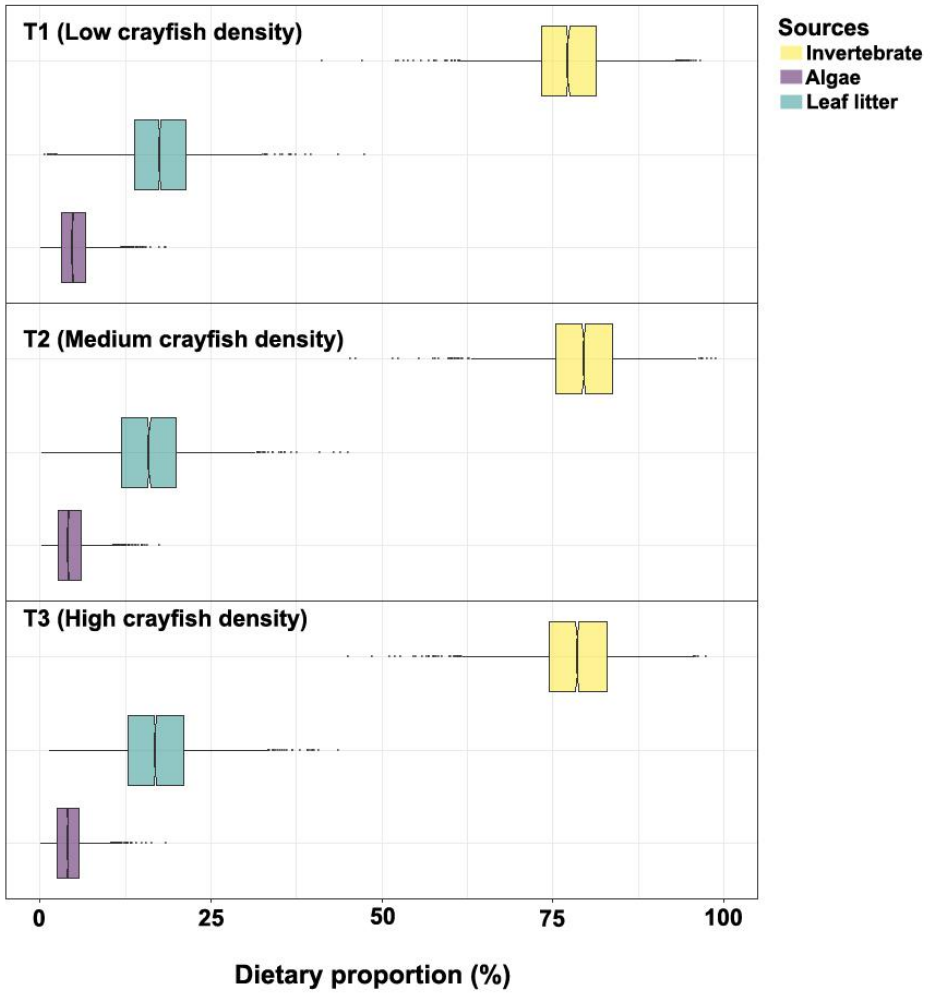


FIGURE 2.10 Boxplots showing comparisons of signal crayfish dietary proportions for different prey sources in different treatment groups (T_1, T_2 and T_3 are low, medium and high crayfish density treatments respectively). Midline within the box is the median; upper and lower limits of the box represent the third and first quartile (75th and 25th percentile) respectively. Points are individual enclosure data.

In the case of bullhead, SIMM results clearly showed changes in dietary proportions of different prey items across groups (Table 2.10, Figure 2.11). Consumption of predatory invertebrates by bullhead declined with increasing density of signal crayfish in the enclosures (Figure 2.12). In control enclosures (C_2) predatory invertebrates comprised about 50% of the bullhead diet whereas it reduced to about 40% for bullhead in enclosures with the highest signal crayfish density (T_3; Table 2.10). However, on the

other hand, increased consumption of chironomid larvae is indicated by the model; it was 37.9% in the bullhead control group (C_2) but increased to 44.9 – 49% in treatment groups with signal crayfish (Table 2.10, Figure 2.12).

TABLE 2.10 Quantification of bullhead diets belonging to different groups (C_2, bullhead only; T_1, T_2 and T_3 are low, medium and high crayfish density treatments respectively), based on Stable Isotope Mixing Model (SIMM). Grazers and shredders group includes Heptageniidae, Baetidae, Gammaridae and Hydropsychidae.

Groups	Bullhead diets (%, mean ± SD; 95% CI)		
	Chironomids	Grazers & shredders	Predatory caddis
C_2	37.9±0.02 (34.1–41.6)	12.3±0.02 (8.3–16.7)	49.8±0.01 (47.8–51.7)
T_1	49.0±0.03 (42.6–54.1)	6.6±0.03 (1.7–13.0)	44.4±0.01 (41.4–47.1)
T_2	44.9±0.03 (38.1–49.6)	10.9±0.03 (5.2–16.7)	44.2±0.01 (43.1–47.2)
T_3	46.3±0.03 (40.4–52.3)	13.0±0.03 (7.2–18.9)	40.7±0.01 (38.5–42.8)

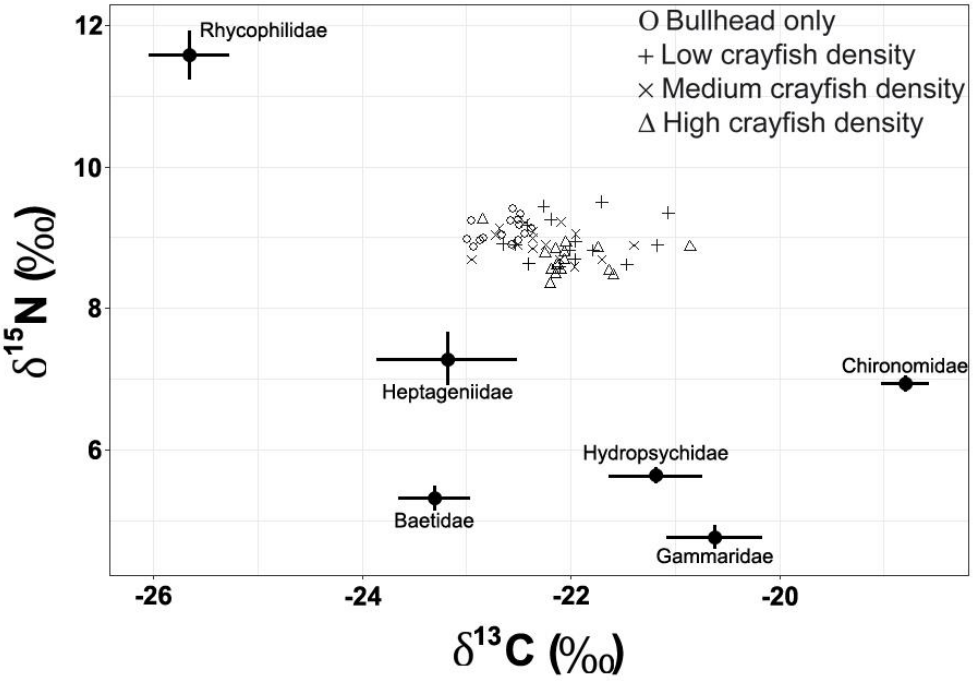


FIGURE 2.11 Isospace plot for three experimental groups of bullhead based on stable isotope signatures of carbon and nitrogen and their prey components in the enclosures. Data for prey groups are represented as mean and standard deviation.

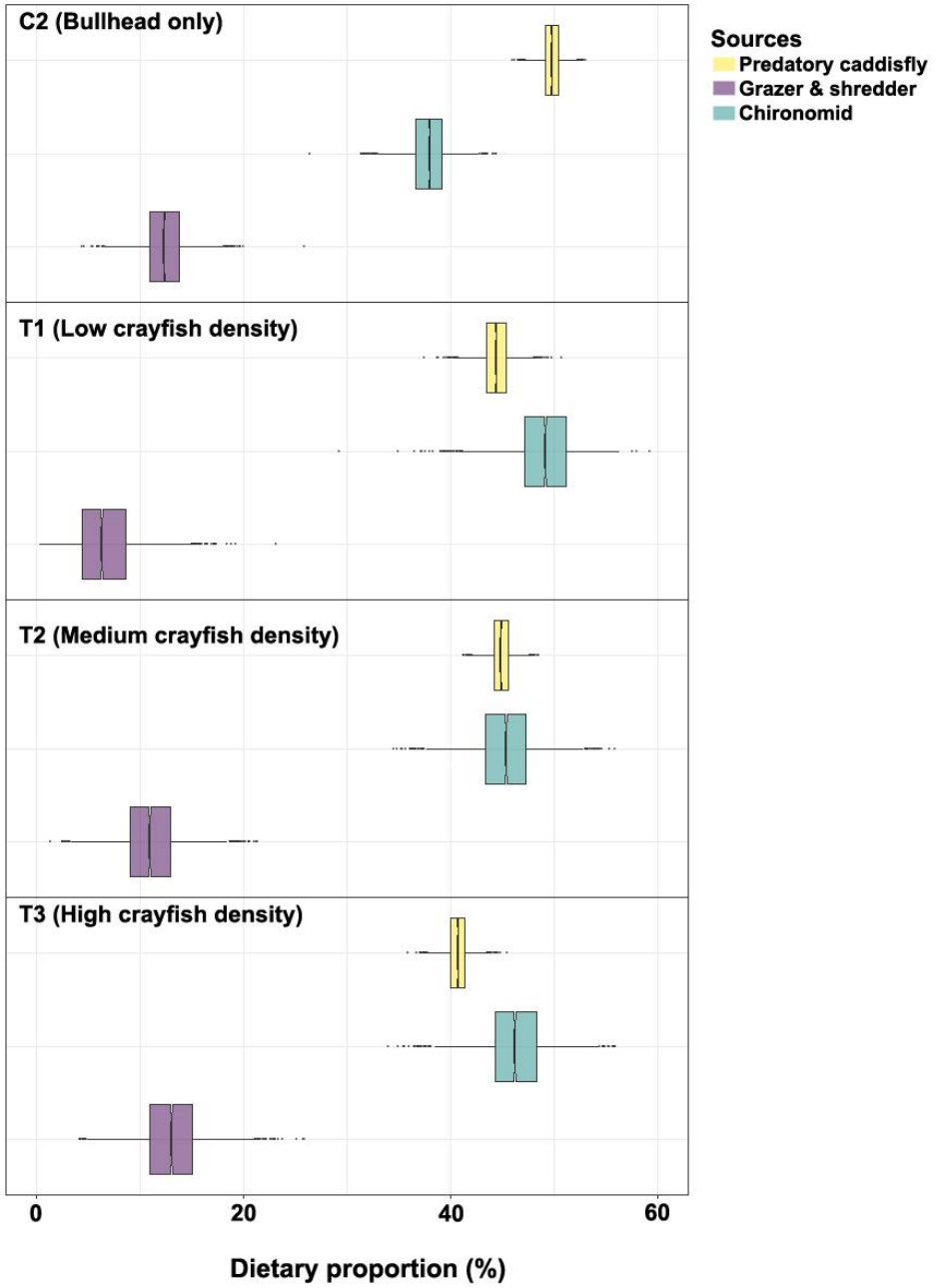


FIGURE 2.12 Boxplots showing comparisons of bullhead dietary proportions for different prey sources in different experiment groups (C_2, bullhead control; T_1, T_2 and T_3 are low, medium and high crayfish density treatments respectively). Sources are chironomids (Chironomidae), grazers and shredders (Gammaridae, Hydropsychidae, Heptageniidae and Baetidae) and predatory caddis (Rhycophilidae). Midline within the box is the median; upper and lower limits of the box represent the third and first quartile (75th and 25th percentile) respectively. Points are individual enclosure data.

2.3.4 Algal standing stock

The highest chlorophyll-*α* level (reflective of algal standing stock) was recorded in the first control group (C_1) with no crayfish and bullhead (mean ± SD: 13.1 ± 2.7 mg ml^{-1}). The lowest amount (1.1 ± 0.4 mg ml^{-1}) was recorded in T_3 with the highest density of crayfish (Figure 2.13). Nested ANOVA results showed significant differences in chlorophyll-*α* between groups ($F = 41.3$, $P < 0.001$). Post-hoc tests confirmed significant differences between control and treatment groups (Table 2.11, Figure 2.13). However, no significant difference was recorded between the two control groups (C_1 vs. C_2). Small to large effect sizes (Hedges' *g*, 0.31 to 3.42) were found between all control and treatment comparisons which indicated strong influences of crayfish treatments on algal standing stock (Table 2.11, Figure 2.14).

TABLE 2.11 Comparison of chlorophyll-*α* content between groups (C_1, control without fish and crayfish; C_2, bullhead control; T_1, T_2 and T_3 are low, medium and high crayfish density treatments respectively), obtained through nested ANOVA post-hoc and effect size (Hedges' *g*) tests.

Groups	*P*-value	Effect size[1]	95% CI
C_1 vs. C_2	0.839	0.26 (S)	–1.18 to 1.70
C_1 vs. T_1	**<0.001**	2.60 (L)	–0.47 to 5.67
C_1 vs. T_2	**<0.001**	3.42 (L)	–0.003 to 6.85
C_1 vs. T_3	**<0.001**	2.03 (L)	1.31 to 2.76
C_2 vs. T_1	**<0.001**	0.31 (S)	0.12 to 0.50
C_2 vs. T_2	**<0.001**	2.04 (L)	–0.31 to 4.40
C_2 vs. T_3	**<0.001**	2.85 (L)	0.05 to 5.65
T_1 vs. T_2	0.078	1.51 (L)	–0.29 to 3.32
T_1 vs. T_3	**<0.001**	4.59 (L)	0.15 to 9.03
T_2 vs. T_3	0.205	1.65 (L)	–0.34 to 3.64

[1]Effect size (Hedges' *g*): L, large; S, small

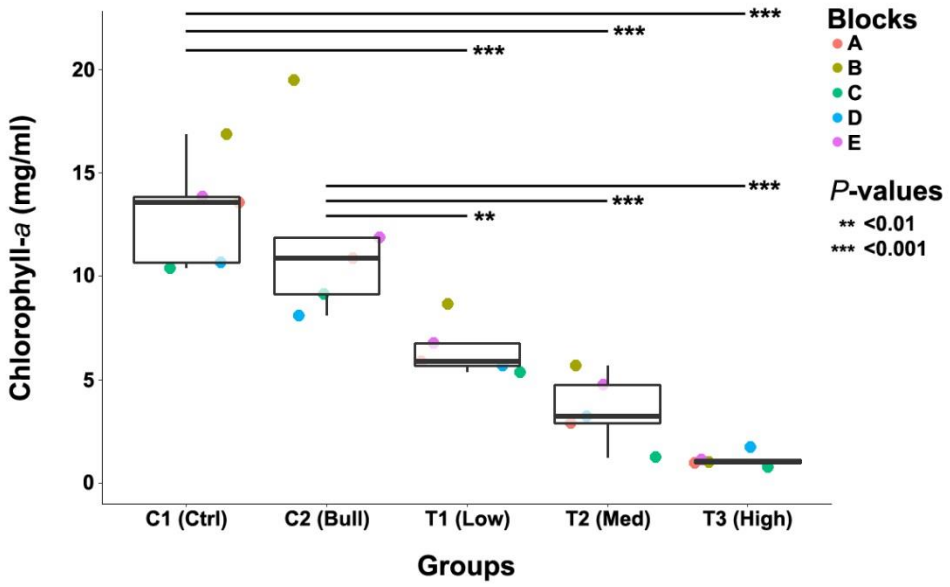


FIGURE 2.13 Chlorophyll-*α* production in different control and treatment groups (C_1, control without fish or crayfish; C_2, bullhead control; T_1, T_2 and T_3 are low, medium and high crayfish density treatments respectively) and comparisons between important groups of interest. Midline within the box is the median; upper and lower limits of the box represent the third and first quartile (75th and 25th percentile) respectively. Points are individual enclosure data.

2.3.5 Leaf-litter decomposition

An opposite trend of chlorophyll-*α* levels was recorded for leaf litter decomposition in enclosure groups in which the highest and lowest rate of daily leaf litter loss were recorded in T_3 (high density treatment; 0.042 ± 0.005 g) and C_1 (no fish and crayfish; 0.026 ± 0.004 g) groups (Figures 2.15 & 2.16). Nested ANOVA results showed a significant difference in the daily loss of leaf litter between experimental groups ($F = 8.0$, $P < 0.001$). Significant variation between control and treatment groups was revealed through post-hoc test (Table 2.12, Figure 2.15). There was no significant difference in the daily rate of leaf litter loss between the two control groups. Effect size analyses revealed large effect sizes (Hedges' *g*, –2.84 to –1.27) between control and treatment group comparisons which, again like

chlorophyll-*α* levels, indicated strong influences of study animals on the loss of leaf litter (Table 2.12, Figure 2.16).

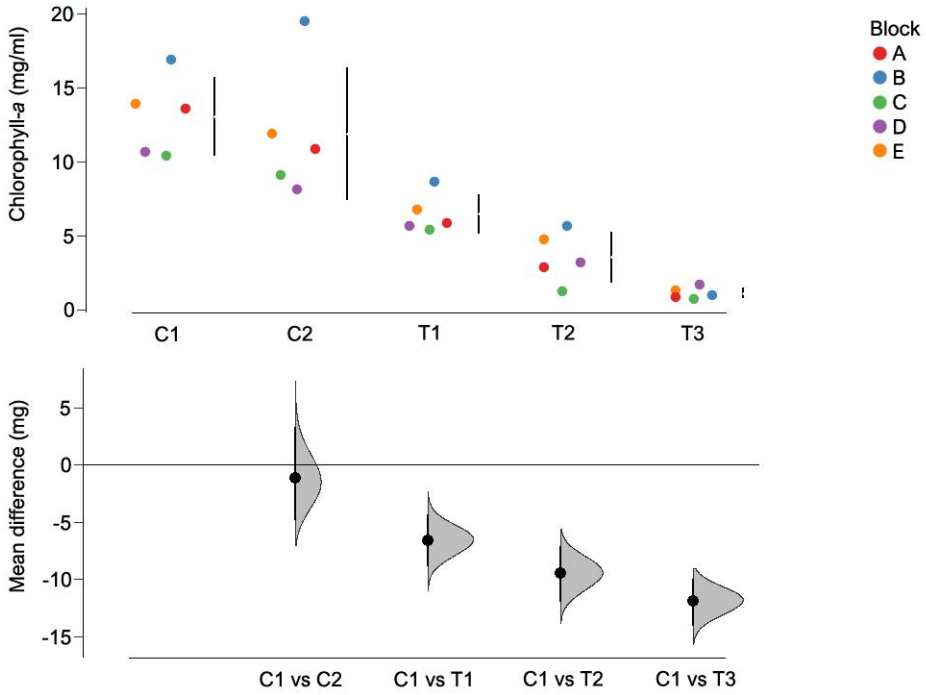


FIGURE 2.14 Impact of signal crayfish, in terms of effect sizes, on chlorophyll-*α* levels in different groups (C_1, control without fish or crayfish; C_2, bullhead control; T_1, T_2 and T_3 are low, medium and high crayfish density treatments respectively), compared to first control (C_1). Dots and vertical bars next to dots (on the right of each group) in the upper panel represent data points and mean (± SD) error bars. Mean values are indicated by the gaps in the lines. Lower panel represents the mean difference (the effect size) and its 95% confidence interval (95% CI) as a point estimate and vertical bar respectively.

TABLE 2.12 Comparison of leaf litter loss rates between groups (C_1, control without fish or crayfish; C_2, bullhead control; T_1, T_2 and T_3 are low, medium and high crayfish density treatments respectively), obtained through nested ANOVA post-hoc and effect size (Hedges' *g*) tests.

Groups	*P*-value	Effect size[1]	95% CI
C_1 vs. C_2	1.00	–0.01 (N)	–0.55 to 0.53
C_1 vs. T_1	**0.048**	–1.27 (L)	–3.43 to 0.89
C_1 vs. T_2	**0.008**	–1.60 (L)	–4.06 to 0.86
C_1 vs. T_3	**<0.001**	–2.82 (L)	–5.31 to –0.33
C_2 vs. T_1	**0.050**	–1.44 (L)	–3.73 to 0.86
C_2 vs. T_2	**0.010**	–1.80 (L)	–4.34 to 0.75
C_2 vs. T_3	**<0.001**	–2.84 (L)	–4.58 to –1.11
T_1 vs. T_2	0.970	–0.24 (S)	–0.68 to 0.19
T_1 vs. T_3	0.388	–0.83 (L)	–2.45 to 0.80
T_2 vs. T_3	0.783	–0.53 (M)	–1.80 to 0.73

[1]Effect size (Hedges' *g*): L, large; M, medium; N, negligible

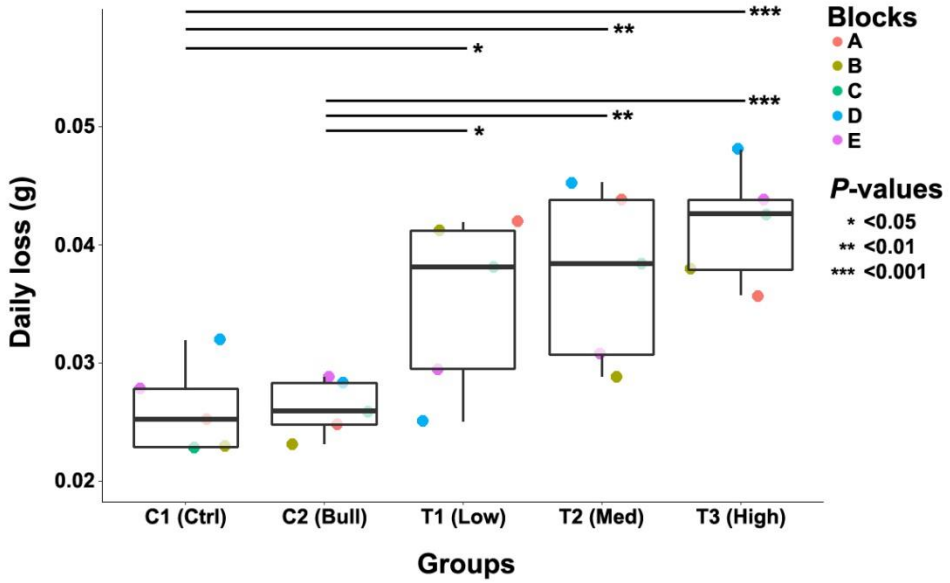


FIGURE 2.15 Loss of leaf litter in different groups (C_1, control without fish and crayfish; C_2, bullhead control; T_1, T_2 and T_3 are low, medium and high crayfish density treatments respectively). Midline within the box is the median; upper and lower limits of the box represent the third and first quartile (75th and 25th percentile) respectively. Points are individual enclosure data.

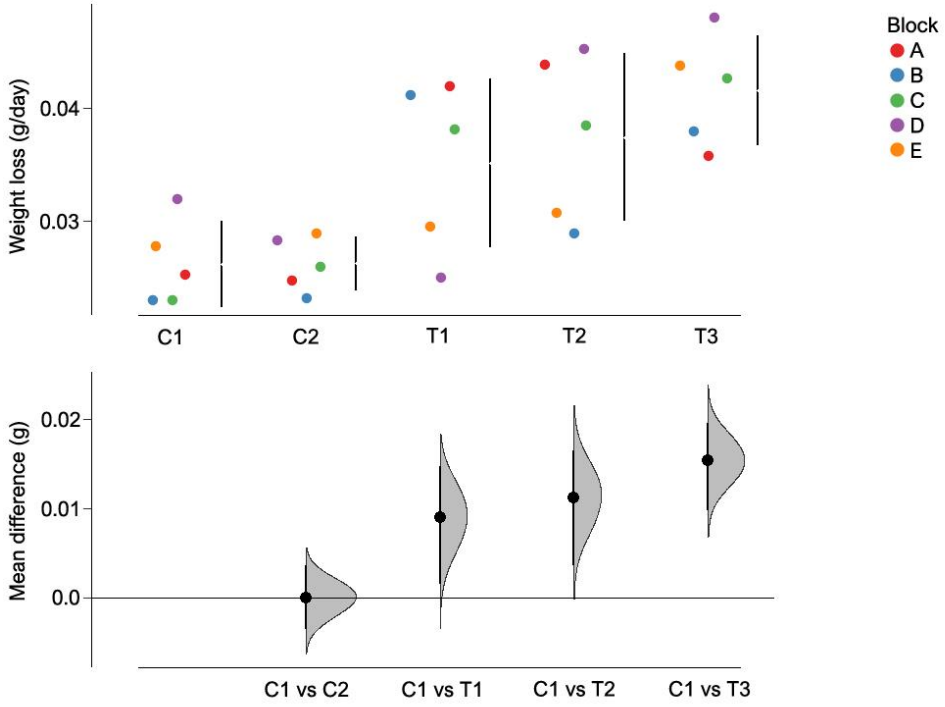


FIGURE 2.16 Impact of signal crayfish, in terms of effect sizes, on leaf litter decomposition in different groups (C_1, control without fish or crayfish; C_2, bullhead control; T_1, T_2 and T_3 are low, medium and high crayfish density treatments respectively), compared to first control (C_1). Dots and vertical bars next to dots (on the right of each group) in the upper panel represent data points and mean (± SD) error bars. Mean values are indicated by the gaps in the lines. Lower panel represents the mean difference (the effect size) and its 95% confidence interval (95% CI) as a point estimate and vertical bar respectively.

2.3.6 Water physico-chemistry

Mean water temperature of the River Lune, recorded continuously by the automatic logger, during the study period was 14.6 ± 1.1°C (*N* = 4338; range: 11.9 – 17.7°C). No high-flow event was recorded during study time and the mean water level was 0.46 ± 0.1 m. LMM results revealed that none of the physico-chemical properties of water varied significantly between experimental groups during the study time (Table 2.13).

TABLE 2.13 Various water quality parameters across control and treatment groups over time duration of study, measured weekly (10:00–12:00) in enclosures during site visits.

Parameters	Mean (± SD)	LMM results	
		F	***P***
Water depth (cm)	23.0 ± 6.4	0.56	0.697
Water temperature (°C)	14.8 ± 0.9	1.15	0.369
Dissolved oxygen (mg L^{-1})	8.6 ± 0.7	1.24	0.342
pH	8.2 ± 0.2	1.74	0.190
Flow velocity (ms^{-1})	0.2 ± 0.1	1.65	0.217

2.4 Discussion

This study shows strong impacts of signal crayfish on stream ecology including negative effects on macroinvertebrate, native benthic fish and important ecosystem processes like breakdown of organic matter and primary production. These impacts are speculated to be sufficient to disrupt the existing food web and could lead to a strong trophic cascade. It is evident from the results that the changes recorded in this study were not because of water quality parameters but due to signal crayfish, as no significant variation was recorded in any of the water quality parameters across enclosure groups.

2.4.1 Impacts on macroinvertebrates

Signal crayfish are well-known for their adverse impacts on macroinvertebrate communities (Nyström *et al.*, 1996; Crawford *et al.*, 2006; Mathers *et al.*, 2016). This was evident in this study where a decreasing trend of macroinvertebrate abundance and taxonomic richness were recorded in relation to increasing signal crayfish density in treatments. As a result, despite no significant differences in macroinvertebrate communities across enclosure treatment categories before introduction of study animals to enclosures, the community differed significantly between experimental groups at the end of experiment.

The highest macroinvertebrate richness and abundance were recorded in first-control enclosures (C_1) with no signal crayfish and bullhead, and these did not change over the course of the experiment. It can be assumed that the absence of direct predation by these two study species (i.e. bullhead and signal crayfish) led to no changes in invertebrate richness, abundance as well as community over time. The density of macroinvertebrates in the first control enclosures (~650 individuals m^{-2}) was lower than the macroinvertebrate density reported in other upland streams (~1100 individuals m^{-2}) (Armitage *et al.*, 1974; Crawford *et al.*, 2006). This may be explaied by the Grassholme Reservoir upstream of the study site as flow regulation can adversely affect invertebrate community (Boon, 1988) and an abundance, similar to the recorded abundance in this study, may be expected (Cowx *et al.*, 1981). The effect of study duration may also be important which may have allowed them insufficient time to colonise fully. Although bullhead are important invertebrate predators in freshwater habitats, they caused no significant change in invertebrate abundance, taxonomic richness and only marginal change in community structure. Bullhead feed primarily on benthic macroinvertebrates (Western, 1969; Mills and Mann, 1983; Woodward *et al.*, 2008). However, their predation at the density considered in this study (five individuals per 1.5 m^2) seems not to have been sufficient to drive changes, as although both richness and abundance reduced to some extent in C_2, these changes were not significantly different from C_1. The 5 mm mesh enclosure design will have facilitated macroinvertebrate drift (at least of smaller instars and taxa) into, and continuous colonisation of, the enclosures and offset the impacts of bullhead predation.

SIMPER outcomes revealed that flattened mayfly larvae (Heptageniidae) contributed the highest proportion (~15%) to the overall difference between before and after communities of macroinvertebrates in treatment enclosures with signal crayfish and bullhead, followed by Chironomidae, Gammaridae Simulidae and Baetidae (the exact order depending upon treatment, see Tables S2.4 – S2.6). Although heptageniid and chironomid changes were not generally significant in controls or treatments, their relative influence is not unexpected because in the River

Lune these families dominated the macroinvertebrate samples. Consistently, Gammaridae were significantly reduced in crayfish treatment groups suggesting a strong effect on the shredder community. SIMPER analyses also showed that the abundance of some of the large invertebrates groups (e.g. Rhyacophilidae and Hydropsychidae) declined in treatment groups and also differed between groups with or without signal crayfish. Previous study with another crayfish *P. clarkii* has revealed crayfish's preference to larger invertebrates (Klose and Cooper, 2012) and thus it is possible that these groups suffered from high predation pressure than other macroinvertebrate groups. Rhyacophilidae and Hydropsychidae are also slow-moving, soft-bodied taxa which may be susceptible to crayfish predation.

2.4.2 Bullhead–signal crayfish interactions

This study showed that signal crayfish can significantly affect bullhead diet without affecting their own trophic position as SIMM results showed that there was almost no change in diet of signal crayfish across groups, even at the highest density. Bullhead growth was affected in a density-dependent fashion by signal crayfish and at the highest crayfish density bullhead lost weight. Moreover, bullhead from the high crayfish density treatment group (T_3) occupied a lower trophic niche (= with low $\delta^{15}N$ values) and with higher $\delta^{13}C$ values compared to other groups.

The outcomes of the enclosure experiment and trophic analyses indicate a high level of competition between signal crayfish and bullhead in which bullhead may have had reduced access to preferred high-quality diets and / or the amount they ate was not adequate to maintain normal growth and resulted in reduced or even negative growth at the end of the study. This result agrees with the findings that signal crayfish can be responsible for reduced (= negative) growth rate and gut fullness of Paiute sculpin *Cottus beldingi* (Light, 2002, 2005), a close relative to the benthic fish species used in this study (i.e. bullhead). Light (2005) showed that sculpin lost a mean weight of 0.28 g day^{-1} when they were kept with signal crayfish. Interestingly, in habitats where crayfish co-occur with another non-native species, it can avoid interactions with other invasive species (e.g.

reported between invasive Eurasian watermilfoil *Myriophyllum spicatum* and rusty crayfish *Orconectes rusticus*; Maezo *et al.*, 2010).

SIMM results of this study also revealed that bullhead in treatment enclosures (i.e. those with signal crayfish) consumed less large prey occupying a higher position in the food web (e.g. predatory caddis larvae) than those from control enclosures. For bullhead from control enclosures, the contribution of predatory invertebrates such as the caseless caddisfly family Rhycophilidae (but potentially also predatory stoneflies such as Perlodidae and Perlidae which were also present) to the overall diet was ~50% but it reduced to ~40% in treatment groups with increasing density of signal crayfish. Whereas, an opposite relationship was found for Chironomidae wherein bullhead from treatment enclosures are modelled to have consumed more chironomids than those from the control group. This shift from prey occupying a higher position on the trophic levels (e.g. Rhycophilidae) to another prey occupying lower trophic niche (e.g. Chironomidae) resulted in bullhead from the medium and high density treatments occupying lower trophic positions than other groups, especially when compared to bullhead control group (C_2).

A similar explanation may be applied to higher $\delta^{13}C$ values recorded in bullhead tissues from the high signal crayfish density group. Increasing consumption of prey with a higher carbon isotope value (e.g. Chironomidae) than those with lower values (e.g. Rhyacophilidae) may have resulted in a higher $\delta^{13}C$ values in the tissues of bullhead from the high signal crayfish density group. A study with the midwater fish chub *S. cephalus* (Wood *et al.*, 2017), common in lowland rivers, showed that young-of-year (YoY, 0+) chub at non-native signal crayfish invaded rivers exhibit a significantly lower growth rate compared to those from uninvaded sites. By contrast, large chub from crayfish invaded sites showed better growth rates than large individuals from uninvaded sites, indicating positive effects of signal crayfish on large individuals, interpreted as being due to crayfish becoming a key part of the diet of larger chub. Unlike chub, bullhead have a small ultimate size at adulthood and can only predate the smallest (mostly YoY) crayfish that were not recorded in this study. In this study all bullhead were adult and moderately large in size but still suffered

from reduced or negative growth over time. This may be due to differences in niche and mode of locomotion between crayfish and finfish species considered in the study; chub is a moderately fast-swimmer and occupies the midwater niche of a habitat whereas bullhead are slow-moving bottom dwellers and depend on refuge within the habitat, similar to crayfish (Freyhof and Kottelat, 2007). Thus the present findings are in accordance with the hypothesis of this study that the impacts of signal crayfish will be higher on species occupying similar niche.

Significantly lower stable isotopic values were recorded in bullhead liver and signal crayfish hepatopancreas tissues than muscle. It should be noted that although the turnover rate is faster in tissues like liver or hepatopancreas than muscle, isotopic enrichment may be lower in these tissues because of high lipid content (Tieszen *et al.*, 1983; Chen *et al.*, 2012) as revealed in this study. Findings of the other studies, based on fishes, have revealed than muscle tissue is the most consistent proxy for stable isotopes (e.g. $\delta^{13}C$) (Hesslein *et al.*, 1993; Pinnegar and Polunin, 1999; Chen *et al.*, 2012).

Two bullhead were not recovered from the high-density treatment; their fate is unknown but it is very unlikely that they escaped as the enclosures had no holes upon retrieval and all crayfish were recovered. It is therefore likely that they died, but the role of crayfish in this regard is unknown. The capability of signal crayfish to attack and consume bullhead has been reported earlier (Guan and Wiles, 1997). Records of injuries on amphibians (California newt) due to direct attack from non-native crayfish are available (Gamradt *et al.*, 1997). They can also predate on eggs and larvae of various amphibian species including newts, frogs and toads and can result in cent per cent mortality (Axelsson *et al.*, 1995; Gamradt and Kats, 1996). Direct predation on large invertebrates including native river shrimp (*Atyaephyra desmarestii*) by invasive crayfish was also reported in Portugal (Banha and Anastácio, 2011).

2.4.3 Impact on ecosystem processes

Significant effects on two important processes of ecosystem (i.e. leaf litter breakdown and algal growth) were evident in this study. A strong negative

relationship was recorded between signal crayfish density and leaf litter breakdown. Compared to both control groups, loss of leaf litter was significantly higher in signal crayfish containing enclosures, even in the group with the lowest number of crayfish (T_1). However, as abundance of key shredders like *Gammarus* was greatly reduced in the high density crayfish treatment it may be expected that it would lead to trophic cascade in the system, which could result in reduced leaf litter processing or loss in the high crayfish density treatment. But the opposite results were recorded in this study which may be due to direct effects of signal crayfish (Doherty-Bone *et al.*, 2018).

It may be assumed that this could be due to a density effect of crayfish as studies showed that signal crayfish extensively feed on leaf litter or detritus in a riverine habitat and can contribute 67.5% to the overall food of crayfish (Mason, 1975; Stenroth and Nyström, 2003). Contribution of leaf litter to the overall diet of crayfish was much less in the present study which may be due to the limited availability of leaf litter in the enclosure as the mesh of enclosures may have restricted outside leaf litter from entering. SIMM outcomes suggested an unchanged proportional consumption of leaf litter by crayfish in enclosures. This would result in increasing foraging pressure on leaf litter with an increasing density of crayfish in the enclosure and processing of available leaf litter at a higher rate, as happened in this study. This assumes crayfish could directly access leaf litter from the experimental packs, and although they could not enter the packs, it seems likely they could access it using their maxillipeds and chelipeds. This result is also in accordance with the fact that crayfish are an active shredder and can play an important role in processing leaf litters in ecosystems (Usio and Townsend, 2001).

In this way, crayfish can play in important role in breaking down leaf litter or similar organic matter in stream ecosystems and this is expected to be beneficial for collector-gatherer macroinvertebrates including Chironomidae and Oligochaeta (Huryn and Wallace, 1987). Nonetheless, it has been revealed that a low density of invasive crayfish (*Orconectes meeki meeki*) can effectively reduce the biomass of benthic chironomids (Ludlam *et al.*, 2015). Therefore, it is very difficult to predict the impacts of

invasive crayfish on ecosystem components as their role depends on a range of associated factors (Huryn and Wallace, 1987; Klose and Cooper, 2012). For example, in a study conducted in outdoor fiberglass tanks, mixed results were obtained in which the presence of signal and red-swamp crayfish increased the rate of leaf litter decomposition but it decreased in tanks with virile and Turkish crayfish (Jackson *et al.*, 2014). The outcome of the present study is in accordance with Jackson *et al.* (2014) as signal crayfish significantly reduced leaf litter in both studies. Bullhead may also play an important role in slowing down the organic decomposition process by preying on shredder macroinvertebrates like Chironomidae and Baetidae (Woodward *et al.*, 2008) but this was not found to be important in the current study at the bullhead densities used. At densities of signal crayfish and bullhead that do occur in northern England upland streams, the effects of signal crayfish as an active shredder is much higher and is enough to significantly accelerate organic matter decomposition, even in presence of a species playing an opposite role.

SIMM results indicated that within experimental enclosures used in this study, macroinvertebrates constituted the major portion of signal crayfish diet which is in contrast with the findings of Bondar *et al.* (2005) who reported that the amount of macroinvertebrate formed a minor proportion of signal crayfish gut and it is not related to crayfish density. One possible explanation may be restricted access of detritus matter within enclosures from outside that might have prevented signal crayfish from consuming a greater amount (Ludlam *et al.*, 2015). However, Whitledge and Rabeni (1997) reported that 30 – 50% of the crayfish production is derived from direct consumption of animal matter which supports of the results obtained in this study. No crayfish sex effects on isotopic signatures were found in this study and suggests there is no difference in foraging and diet between the sexes of signal crayfish.

Another important ecosystem process, algal growth (measured as an index by chlorophyll-*α*), was negatively affected by the signal crayfish density. Compared to both control enclosure groups (C_1 and C_2), chlorophyll-*α* levels were significantly lower in enclosures with signal crayfish. Again, like leaf litter, SIMM suggested that algae provided a

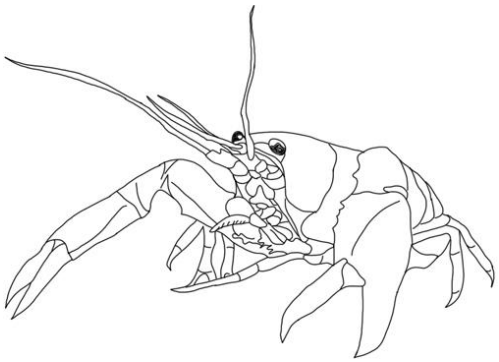
relatively constant contribution to signal crayfish diet across density treatment groups. Therefore, a lower amount of algal production can be expected in environments with a high density of crayfish, as revealed in this study. This result is expected in habitats with crayfish as they can negatively affect the abundance of algal cells including diatoms (Keller and Ruman, 1998). Although, due to the feeding nature of crayfish, it could be expected that slow moving macroinvertebrate taxa including common grazers would be reduced in abundance (Mathers *et al.*, 2016), as happened in this study as well, partially releasing algae from grazing pressure that may create trophic cascade and could lead to increased algal growth. Similar impacts of bullhead on grazing macroinvertebrates are also expected (but note, bullhead also fed extensively on invertebrate predator taxa) and this might also increase algal biomass through decreasing the abundance of grazers (Dahl, 1998). But, both the abundance of grazers and algal growth were negatively affected in enclosures with signal crayfish indicating a board spectrum of impacts over multiple components of the ecosystem. This indicates that direct grazing effects of signal crayfish were more important than indirect trophic cascade in habitats with high crayfish density. Studies (e.g. Momot, 1995; Ludlam *et al.*, 2015) have also shown that crayfish can impact plant biomass negatively even at a low density which is in agreement with the findings of this study. However, if intraspecific competition is high or resources are limiting, omnivores may alter their diets (Svanbäck and Persson, 2004; Bondar *et al.*, 2005) and therefore it may be assumed that the increasing density of signal crayfish will affect algal growth, along with other common prey items.

For signal crayfish, it has been shown that this species does not undergo ontogenetic niche shifts in streams (Bondar *et al.*, 2005) and there is no effect of body size or seasons on isotopic signature values (France, 1996; Stenroth *et al.*, 2006). Therefore, it is likely that the results of stable isotope analyses of this study would effectively represent signal crayfish of all sizes in streams of the type studied, while acknowledging that the mesocosms used are not true representations of the stream environment. Nevertheless broadly similar outcomes may be expected with signal crayfish under similar study environments.

2.5 Conclusions

This study adds important information to literature regarding impacts of signal crayfish on various ecosystem components. It effectively showed the magnitude of impacts of signal crayfish at different densities on macroinvertebrates, a native benthic fish species and various processes of an invaded ecosystem and all these justify its role as a keystone species. Evidence from this study suggested that signal crayfish, at the densities and conditions studied, exhibited more direct effects on the benthic stream ecosystem than were evident from potential trophic cascade processes. Signal crayfish did not shift its diet to a great extent but forced bullhead to shift from what is assumed to have been a high-quality diet towards a lower-quality diet, contributing to reduced growth performance. This indicates interspecific competition was occurring.

It is believed that this study would provide a reliable prediction about the impacts of signal crayfish at different densities on various typical ecosystem components of an invaded upland stream habitat in England. It may be expected that bullhead populations will suffer from food availability with increasing densities of signal crayfish in study areas that could lead to reduced fitness and contribute to extirpation (Momot, 1995). However, with consideration of the present findings, it would not be a surprising fact if invasive signal crayfish continue to modify the ecology of invaded habitats, such that bullhead populations may decline in the long run as this transformation of habitat may become unfavourable to them (Larson *et al.*, 2019). Finally it is recommended that long-term studies should be carried out in streams with varying densities of signal crayfish to test the results of this study. The suggested study should effectively represent both before and after scenarios of signal crayfish invasion for any invaded stream and invaded streams should also be compared to uninvaded control streams over the similar time period to better quantify the changes.



Chapter Three

DETERMINING THE DRIVERS OF INVASIVE SIGNAL CRAYFISH DISPERSAL

Summary

Biological invasion is partly responsible for the decline in native taxa globally and a better understanding of the mechanisms underpinning invasion dynamics is needed. Increasing emphasis has been placed on the role that 'personality' may have during invasion. Few studies have investigated personality in relation to invasion and mostly they have been in controlled environments, rather than the wild. The influence of other factors with the potential to affect invasion has rarely been considered.

Here, dispersal, a key component of biological invasion, of signal crayfish *Pacifastacus leniusculus*, was measured in relation to behavioural traits indicating boldness and exploration, as well as crayfish size, sex, population density and habitat. Field experiments were carried out in fully-established (FE), newly-established (NE) and invasion front (IF) sites of two northeast England streams.

Crayfish exhibited strong consistency in behavioural traits over time (ICC 0.39 – 0.94, all $P \leq 0.022$) and formation of context-dependent behavioural syndromes. However, their roles in dispersal varied across sites and were linked to refuge availability in habitats with medium to high crayfish density. In FE, bold and exploratory individuals dispersed less ($P < 0.001$) but an opposite trend was recorded in NE and IF (both $P < 0.05$). Climbing tendency in trials also significantly positively affected dispersal in NE and IF.

This study concludes that a better understanding of animal invasions can be achieved by a fuller knowledge of the interplay between behaviour, ecology and habitat complexity.

Keywords: Signal crayfish, animal personality, biological invasion, dispersal, upland rivers

3.1 Introduction

Human activities, in the form of increasing trade, tourism and population expansion have facilitated the spread of non-native species outside their natural range, both intentionally and unintentionally (Levine and D'Antonio, 2003; Hulme, 2009; Bellard *et al.*, 2016; Dawson *et al.*, 2017). Non-native species, especially invasive ones are one of the major causes of biodiversity loss worldwide (Chapter One; Naeem *et al.*, 2012; Caffrey *et al.*, 2014; Veale *et al.*, 2015). Over the last few decades in biological invasion research, priority has been given to exploring the biological and ecological characteristics of non-native species underlying their ecological impacts and invasiveness (Facon *et al.*, 2006; Gurevitch *et al.*, 2011). More recently intraspecific variations have been emphasised (Bolnick *et al.*, 2003, 2011) and applied to studies of biological invasions to explain different aspects of invasion (e.g. Duckworth and Badyaev, 2007; Cote, Fogarty, *et al.*, 2010; Cucherousset *et al.*, 2013).

Within populations individuals often differ consistently in their behaviours across time and contexts (Wilson, 1998; Sih, Bell, and Johnson, 2004; Sih, Bell, Johnson, *et al.*, 2004; Réale *et al.*, 2007). There is an increasing recognition of this inter-individual behavioural difference within animal populations (i.e. personality) in recent times (Réale *et al.*, 2010; Wolf and Weissing, 2012; Mittelbach *et al.*, 2014). Behavioural variations have been studied in a wide range of animal groups including mammals (Morton *et al.*, 2013), birds (Verbeek *et al.*, 1996), reptiles (Herzog *et al.*, 1989), amphibians (Halliday, 1976), fishes (Cote, Fogarty, *et al.*, 2010), insects (Niemelä *et al.*, 2013) and others (see Gosling, 2001 for an extended list). Such inter-individual differences, often regarded as individual behavioural types or personalities (Wolf and Weissing, 2012), play a key role in determining how individuals interact with their ecosystem (Juette *et al.*, 2014) and consequently, can be significant drivers of population dynamics, with impacts on a range of life history stages (Duckworth and Badyaev, 2007; Conrad *et al.*, 2011). For example, bolder individuals are likely to be more willing to venture further, or emerge from shelter sooner than shyer individuals, and consequently can be more successful in resource acquisition, and ultimately fitness outcomes

(Sundström *et al.*, 2004; Ward *et al.*, 2004; Smith and Blumstein, 2008). However, such behaviour comes at a price, with bolder individuals often taking greater risks, exposing themselves to increased probabilities of predation or disease, potentially resulting in increased mortality (Magnhagen, 1991; Lind and Cresswell, 2005; Biro *et al.*, 2006; Stamps, 2007; Barber and Dingemanse, 2010; Kortet *et al.*, 2010). These fundamental impacts of personality on how individuals utilise their environment are increasingly being recognised as important considerations in management and conservation of natural populations (Cote, Fogarty, *et al.*, 2010; Juette *et al.*, 2014; Hirsch *et al.*, 2017). However, personality traits are often correlated with each other and form a 'behavioural syndrome' (Wolf and Weissing, 2012).

Dispersal, a key characteristic of any population, may also be influenced by animal personality (Cote *et al.*, 2011; Quinn *et al.*, 2011; Brodin *et al.*, 2013) and this process is particularly important in range expansion of a species. The potential impacts of animal personality on invasion dynamics, particularly dispersal of non-native species has been identified as a potentially important driver of invasion success (Duckworth and Badyaev, 2007; Cote, Fogarty, *et al.*, 2010; Malange *et al.*, 2016).

Several personality traits have been recognised for their role in dispersal or range expansion. For example, enhanced exploration and activity is often linked with increased fitness and thus, more exploratory / active individuals are expected to play a key role in range expansion by dispersing further (reviewed in Juette *et al.,* 2014). Boldness can also positively affect the spread of a population (Chapple *et al.*, 2012). Recent literature has suggested that the presence of individuals that are bold, more asocial and active help invasive populations to spread further (Chapple *et al.*, 2012) and personality-biased dispersal could be expected on the invasion front (Duckworth and Badyaev, 2007; Cote, Clobert, *et al.*, 2010). Thus, personality-dependent dispersal might be an important factor in determining success of biological invasion but only a limited number of studies have focused on this issue so far (e.g. Duckworth and Badyaev, 2007; Cote, Clobert, *et al.*, 2010; Hirsch *et al.*, 2017). However, the role of personality in determining invasiveness can be unclear (Groen *et al.*,

2012). Some studies suggest that individuals on the invasion front, those leading the range expansion, are more aggressive or active than their counterparts inhabiting established areas (e.g. observed in western bluebird *Sialia mexicana*, Duckworth and Badyaev, 2007; in cane toad *Chaunus marinus*, Urban *et al.*, 2008). However, the opposite trend has also been reported with studies showing that less aggressive individuals lead the invasion (e.g. in the ant *Linepithema humile*, Suarez *et al.*, 1999; in mosquitofish *Gambusia affinis*, Cote, Fogarty, *et al.*, 2010; and the cichlid fish *Hemichromis letourneuxi*, Lopez *et al.*, 2012). These seemingly contradictory results suggest that invasion dynamics may also be influenced by others factors in complex interactions with personality traits (e.g. Dingemanse and Wolf, 2010; Dingemanse and Réale, 2013; Weiss, 2018).

Many existing studies on the role of behavioural types in invasion by non-native species (e.g. Suarez *et al.*, 1999; Cote, Clobert, *et al.*, 2010; Lopez *et al.*, 2012) were carried out under controlled laboratory environments. Their results may not be representative of processes in the natural environment, where context may vary between species, populations and personality types and over time (Archard and Braithwaite, 2010). Therefore, more empirical work is required to understand the complexity of range dynamics with respect to personality in the wild (Sih, Bell, and Johnson, 2004; Holt *et al.*, 2005) both within and between populations (Quinn *et al.*, 2011). In aquatic and other animal populations, evidence of temporally consistent, cross-contextual patterns of personality-dependent dispersal remains rare (Liedvogel *et al.*, 2013).

Movement patterns also govern the way animals use habitats, interact with conspecifics, avoid predators and obtain food (Wilson *et al.*, 2015). Animal taxa that disperse by walking along constrained habitat corridors may be obstructed in doing so, for example upstream movement of crayfishes may be inhibited by cascades and bedrock sills (Bubb *et al.*, 2006b, 2009). Willingness to climb, a trait that has received less attention in personality studies, could be an important factor for some taxa such as walking arthropods, including crayfishes (Rice *et al.*, 2012), enabling them to traverse obstacles and facilitate dispersal and invasion.

In this study five behavioural traits likely to be indicators of boldness and exploration were measured in signal crayfish *Pacifastacus leniusculus*. The role of boldness and exploration on dispersal tendencies was examined in the wild across different contexts represented by three distinct phases of invasion in two upland streams; a fully-established population, a newly-established population and at the invasion front. In addition, the influences of physical characteristics of crayfish and habitat characteristics on dispersal were also evaluated, to better understand underlying invasion dynamics in relation to behavioural traits and context. A previous study with noble crayfish (*Astacus astacus*) showed that boldness was consistent over time and context for crayfish (Vainikka *et al.*, 2011). But it remains unclear whether traits indicative of boldness, along with other behaviours, can affect dispersal, as predicted for aquatic non-native fish taxa (e.g. mosquito fish, Cote, Fogarty, *et al.*, 2010; round goby *Neogobius melanostomus*, Hirsch *et al.*, 2017). Therefore, this study investigated whether behavioural traits indicative of boldness and exploration in signal crayfish were consistent over time, whether different traits constituted a behavioural syndrome, and whether these patterns were consistent across geographical locations. The pattern of dispersal in this invasive species was investigated in relation to these behavioural traits. Finally, the relative importance of behavioural, physical, habitat and population characteristics driving the dispersal of invasive crayfish in the different stages of the invasion process, was analysed, thus testing whether personality impacts on dispersal were independent or dependent on ecological and environmental context.

The hypotheses of this study were (i) invasive signal crayfish show consistency in behaviour traits over time and these are correlated to each other and form a behavioural syndrome; (ii) signal crayfish dispersal in the wild is significantly influenced by its behavioural traits; and (iii) along with behavioural traits, other important factors (e.g. local population density, refuge availability etc.) are also responsible for signal crayfish dispersal in the wild.

3.2 Materials and methods

3.2.1 Pilot studies

In 2017, between April and June, a total of 60 crayfish were randomly collected on three occasions from the wild (Wilden Beck [54°34'51.1"N 1°59'47.1"W], a tributary of the River Tees) by hand-net searching. On the first occasion, crayfish (*n* = 30) were brought to the laboratory and held in plastic tanks (35 cm long × 21 cm wide × 21 cm high) for up to three weeks at different densities (typically one crayfish per tank, but two tanks with two crayfish and two with three crayfish) with 5 cm gravel/pebble layer (2 – 6.4 mm), 3 – 6 cobbles (6.4 – 64 mm) and shelter/s made of cut PVC pipe of different diameters. Water depth (dechlorinated tap water) was maintained at ~15 cm and continuous oxygen supply by aeration was provided. One small aquarium filter was installed per tank. Crayfish were fed carrot and chicken *ad libitum*. Crayfish were collected, held and experiments performed (here and in section 3.2.2) under DEFRA and University of Durham permissions to Dr. M. Lucas.

After being held for 1 week, pilot trials of behavioural typing were carried out. Eighteen randomly chosen crayfish were tested individually by placing each in an experimental arena (rectangular-shaped white plastic container; 60 cm long × 35 cm wide × 20 cm high). Initially crayfish were placed within a shelter (cut-PVC pipe) which was removed carefully after 5 minutes. The crayfish was allowed to explore freely for 10 minutes, without disturbance, while being video recorded to monitor activity, distance moved, climbing (see section 3.2.2.3). After this time a threat response test, to determine boldness (see section 3.2.2.4) was carried out by touching either the crayfish's rostrum from the front or tail from the rear with a long thin stick, ensuring the experimenter was not visible or casting a shadow over the apparatus. To test the behavioural consistency over time, the same 18 crayfish were assessed again, on week 3 of holding.

On the second and third pilot studies, two similar trials (with *n* = 15 each) were also carried out in the field (Wilden Beck; see the 'main experiment', section 3.2.2, below for details) where they were acclimatised

separately, after collection from the beck, for 3 hours in identical separate tubs. These individuals were tested for determining individual behavioural types following the method described above and later transported to the laboratory and housed in plastic tanks, as described above, for repeat assessments. Of these, 15 crayfish were assessed again on Week 1 and Week 3 under laboratory conditions and this allowed the researcher to determine if individuals' behavioural types differ between field and laboratory tests. All tests were video recoded and analysed following standard protocol (see 'main experiment', sections 3.2.2.3 – 3.2.2.4, below for details).

Individuals tested under laboratory conditions, compared to those tested in the field, were less active and performed less exploration or moved over shorter distances (Appendix II, Table S3.1). A time effect was also noticed between successive tests in the laboratory. In the boldness test, four of 18 crayfish showed no response at all during the test on week 3 but did show responses during previous tests in the field or week 1. These variations may be due to holding conditions in the laboratory that altered affected crayfish behaviour, including the possibility of a habituation-type response. Thus, for the main study the behavioural assessments were carried out in the field. In the laboratory 10 crayfish were marked using Visible Implant Elastomer (VIE; see below, section 3.2.2.5) to assess mark retention over time and all the marks were in place after 4 weeks of holding. Marks were also retained by those crayfish that moulted.

3.2.2 Main experiment

3.2.2.1 Study sites

This study was conducted in Westholme Beck and Thorsgill Beck, two upland streams, both tributaries of the River Tees in northeast England (Figure 3.1). Signal crayfish have invaded these streams from the main Tees channel, and spread upstream. In 2017, experiments were carried out from 7 August to 28 September in Westholme Beck (54°33'26.3"N

1°47'53.0"W) which contains a fully-established subpopulation of crayfish (hereafter, FE) at high density (mean crayfish density ± SD, 2.2 ± 1.9 m^{-2} based on area sampling, see below). This population invaded Westholme Beck between 1995 and 2000 (M.C. Lucas, pers. comm.). In 2018, the study was repeated in Thorsgill Beck (54°31'53.5"N 1°54'46.3"W) between 6 August and 20 September, on a newly established subpopulation of signal crayfish that invaded from the Tees (hereafter, NE; invasion age 7 years; M.C. Lucas, pers. comm.) at lower density (mean density ± SD, 1.1 ± 0.7 m^{-2}). This site also provided the opportunity to sample an invasion front (hereafter IF) with a mean density of 0.25 ± 0.3 m^{-2}.

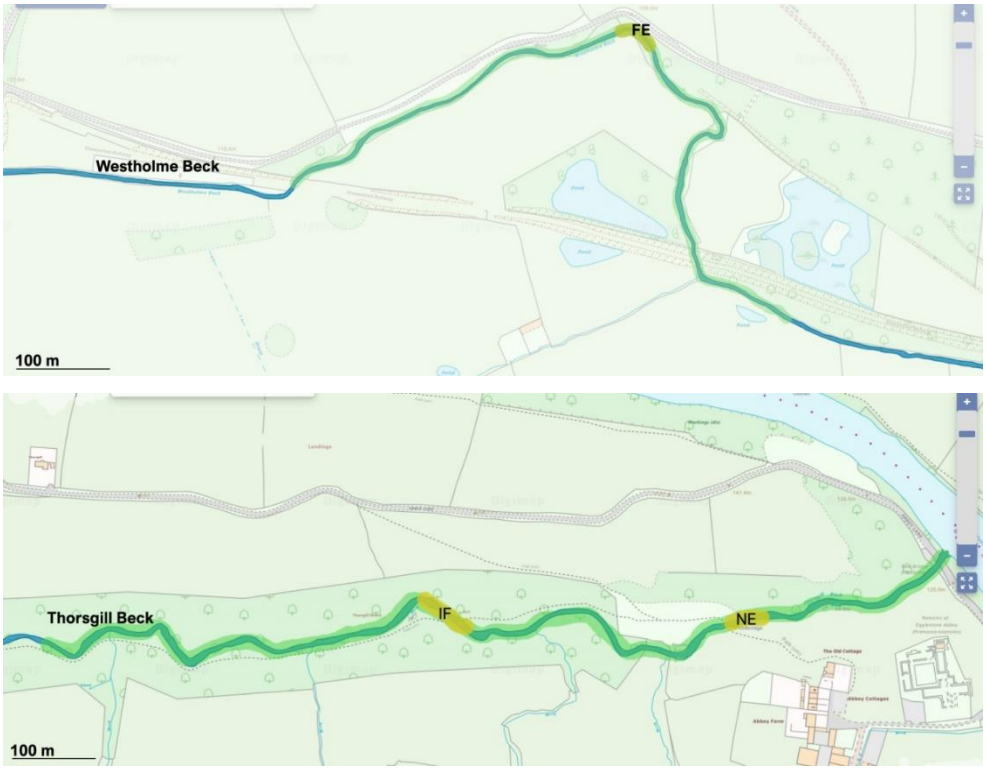


FIGURE 3.1 Map of the study streams (Westholme Beck, above; Thorsgill Beck, below) including three study sites (FE, fully-invaded; IF, invasion front; NE, newly-invaded). Green-shaded areas represent resurveyed reaches of the streams.

Experiments were carried out in summer because this is the time of the year when dispersal and activity of signal crayfish of both sexes is at its highest (Bubb *et al.*, 2002, 2004). In northern England streams, adult female signal crayfish shed their hatchlings before the end of July, most

adults have moulted by then, and mating does not normally commence until the end of September or early October (S. Galib, pers. obs.; Guan and Wiles, 1999; Capurro *et al.*, 2015). The period August - September was chosen for the current experiments as a time when most large juvenile and adult crayfish within a population are foraging and dispersing.

Both streams were initially surveyed to determine the presence, distribution and minimum density of crayfish and habitat structure. The density surveys involved effort- and area-standardised random hand-net sampling of potential refuges suitable for juvenile and adult crayfish, over 1 km reaches of stream, with 30 minutes of searching at 10 locations (~100 m apart) by two experienced crayfish surveyors. This enabled the upstream invasion front to be located in Thorsgill Beck. Immediately after that, for each of NE and IF, six 4 – 7 m long sections within a ~100 m stretch per site were searched to determine the crayfish density more precisely. Both streams contained suitable refuges, primarily in the form of unembedded cobbles and boulders, along with some tree roots and burrows (only in Westholme, within a total of ~2% of 1 km stream length surveyed), for crayfishes. Within the study sites there were no major natural or man-made barriers that could prevent natural dispersal of crayfish either upstream or downstream, though multiple cascades, riffles, and boulder sills, typical of upland streams, existed. The physico-chemical characteristics of the study sites were similar (Table 3.1). Assessment of macroinvertebrate populations by standardised kick sampling at four locations ~50 m apart within each of the FE, NE and IF sites revealed no significant variation in taxonomic richness (linear mixed models, both $P > 0.05$). Brown trout and bullhead were present in both streams. There were no high-flow events during study periods.

TABLE 3.1 Measured habitat/environmental characteristics of the study sites (as Mean ± SD or range). Measurements for width, depth and flow velocity were made during crayfish recapture surveys in September of each year while measures of pH and dissolved oxygen were made from early August to late September.

Characteristics	Westholme Beck	Thorsgill Beck
Wetted width (m)	2.5 ± 0.75	3.7 ± 1.1
Water depth (cm)	13.9 ± 9.4	12.8 ± 5.0
pH	8.1–8.5	8–8.2
Dissolved oxygen (mg L^{-1})	9.4–11.5	9.9–11.38
Flow velocity (m s^{-1})	0.05–0.8	0.05–0.6

3.2.2.2 Collection of signal crayfish

Signal crayfish were collected for study by hand-net searching, targeting larger juveniles and adults (carapace length > 20 mm) since adults and all but the smallest juveniles are capable of upstream movement and may be involved in upstream as well as downstream invasion (Bubb *et al.*, 2006b). In 2017 in Westholme Beck a total of 130 signal crayfish were collected randomly from within the central 60 m of the 1-km study site (Table 3.2). Typically 10 – 20 were caught, processed and returned per study day. In 2018, a total of 180 signal crayfish (Table 3.2) were collected randomly from the IF and NE locations of Thorsgill (90 from each site). The distance between IF and NE centres was 0.5 km and crayfish were collected within the central 45 m and 75 m zones of NE and IF respectively. Signal crayfish with carapace length (CL) <20 mm were not collected as the elastomer mark used in this study (see below) may fragment in smaller individuals (Clark and Kershner, 2006). Newly moulted crayfish were also excluded because they tend to remain in their shelters to avoid predation until their exoskeletons harden (Helfrich and DiStefano, 2009). The location of capture for each crayfish collected was recorded relative to fixed 5-m markers along the river banks, noting river bank and mid-stream features (e.g. distinctive rocks, trees) and by using a GPS (Garmin; accuracy, ±3 m) so that the crayfish could be released at their capture locations. After collection, each crayfish was kept in a separate semi-transparent plastic

tank (35 cm long × 21 cm wide × 21 cm high) containing aerated river water. This was placed in the stream edge for 3 h for acclimatisation prior to behavioural testing, with periodic partial replacement of tank water with fresh stream water. Three cobbles from the stream were provided in each tank for shelter.

TABLE 3.2 Number of total studied and recaptured crayfish, sex ratios and summary statistics for carapace length.

Study stream, year and population	Total crayfish studied			Recaptured crayfish		
	N	**Sex ratio (♂:♀)**	**Carapace length (mm; Mean ± SD and range)**	***N***	**Sex ratio (♂:♀)**	**Carapace length (mm; Mean ± SD and range)**
Westholme, 2017 Fully-established	130	1:1.20	33.1±5.6 (23.0–55.6)	41	1:0.58	31.8±4.6 (23.4–48.2)
Thorsgill, 2018 Newly-established	90	1:0.80	35.7±6.4 (24.5–59.1)	32	1:1.13	35.2±5.7 (24.5–47.5)
Thorsgill, 2018 Invasion front	90	1:0.58	38.6±7.9 (25.9–59.8)	25	1:0.67	39.9±7.8 (31.2–59.8)

3.2.2.3 Assessment of crayfish activity, exploration and climbing tendencies

All behavioural tests were undertaken in the field, on the stream bank, under shade during daytime. The first behavioural tests measured crayfish activity, distance moved, exploration and climbing in a rectangular white plastic tub (52 cm long, 34 cm wide and 25 cm high) with 2×2 cm grid on the bottom, but otherwise devoid of any physical features. This test is essentially a standard open-field test, conducted in an environment that is novel to the crayfish (Yoshida *et al.*, 2005; Cote, Fogarty, *et al.*, 2010). Each test involved transferring a crayfish carefully to a shelter (cut PVC pipe, closed at both ends, attached to a long rod) located in the top-right

corner (by overhead camera view) of the experimental arena. The crayfish was allowed 10 minutes to acclimatise. The shelter was then removed using a rod and the crayfish's behaviour was recorded for 20 minutes by a GoPro video camera (model: Hero 4) located directly above the tank. Stream water was used in the experimental arena during each behavioural assessment and after each recording the arena was washed thoroughly and filled with new water before starting the next behavioural test to avoid any potential effects of odours released by the previous test individual. The experimental arena was surrounded laterally by black curtains during experiments, to minimise visual disturbance.

Recorded videos were exported as image stacks (one frame per second) using the 'ffmpeg' application (version 4.1.3; https://ffmpeg.org) and imported into ImageJ (version 1.52a) where the crayfish's position (position of tip of rostrum; x–y coordinates) was tracked over the 20 min assay. These data were imported into R (R Core Team, 2017) and total distance moved during arena exploration was calculated for each crayfish as cumulative distance moved between each image. Activity was measured as the total number of seconds the crayfish was in motion, by deducting the total duration of time the crayfish remained stationary from the total duration, i.e. 1200 seconds. Exploration was quantified as the percentage of unique grid squares touched by tip of the rostrum during the test. Climbing was defined as when the crayfish was active with its body against the tank wall at an angle of 45 – 90° from horizontal. The total time spent trying to climb up the vertical sides of the tank, usually at the corners, was recorded.

3.2.2.4 Assessment of boldness/threat response

For each individual, after the 20 min exploration/activity/climbing test was completed, the tail of each crayfish was touched gently by using a thin rod from behind to record their response. This test was designed to mimic the threat of a predator in natural environments and crayfish respond in two ways; by either tail-flipping (rapid contraction of the abdomen propelling the crayfish backwards) or by raising their claws (Pintor *et al.*, 2008). Crayfish were categorised into two groups representing 'boldness types', depending

on their responses (Rupia *et al.*, 2016); (i) shy (tail-flipping, retreating individuals), and (ii) bold (individuals who raised their claws). A 'boldness score' was calculated for each individual. For shy individuals, this was based on the combined duration of tail-flipping and subsequent stationary position before they started to move again. For bold individuals, the total duration from initiation of claw raising to when the claws were lowered was used. A bold individual's score (i.e. duration recorded) was denoted as 'positive' and shy individual's score as 'negative' (Karavanich and Atema, 1998), to generate a spectrum of bold-shyness.

3.2.2.5 Measurement of physical characteristics of crayfish and dispersal in natural environment

After the behavioural tests were completed carapace length (using a Vernier scale), body mass (portable pan balance, to 0.001 g), sex and body description (loss or damage to claws, legs and antennae) of each individual were recorded. Crayfish were then marked individually using Visible Implant Elastomer (VIE; Northwest Marine Technology, Inc., Shaw Island, WA, USA) tags, coded by mark location, ventrally, on abdominal somites (Figure 3.2), and released at their original capture locations. VIEs are effective tagging techniques for both adult and juvenile crayfish without affecting crayfish biology and are retained following moults (Clark and Kershner, 2006). Crayfish were photographed, returned to the capture location and left at liberty, without further disturbance for 29.3 ± 4.4 days (mean and SD).

At the Westholme Beck site (FE, 2017), a recapture survey was carried out, commencing 35 days after release of the last crayfish. 1000 m of stream (500 m upstream and 500 m downstream from the midpoint of crayfish releases) was surveyed (hand-net searching; 2 – 3 experienced persons) by dividing the whole study length into 200 sections (each 5 m long) and searching 5-m sections progressively from the midpoint outwards, upstream (US) and downstream (DS). All likely, accessible wetted refuges were searched thoroughly. Although the method contains bias in that, like most crayfish sampling methods, the smallest crayfish are undersampled, it provides a standardised, rapid method, effective in

shallow-upland streams. Unlike trapping, it is relatively non-size and sex-selective; trapping is likely also to bias towards exploratory behaviour types, which was important to avoid in this study (Wutz and Geist, 2013). Densities recorded per section are minimum estimates as, inevitably, some crayfish are inaccessible within tree roots or other refuges. On any given day, sampling progressed in an upstream direction to ensure good search visibility and capture efficiency due to disturbed sediment. Surveying was continued outwards from the centre of the reach until no marked crayfish were captured in outer 300-m zones (Figure 3.1).

FIGURE 3.2 Ventral view of a female signal crayfish showing Visible Implant Elastomer (VIE) codes on abdominal somites.

Resurveying took two weeks. All crayfish captured in each 5-m section were counted, measured, sexed and inspected for presence of a VIE tag. Each recaptured crayfish was photographed, identified, reweighed, measured and limb loss status recorded. The dispersal direction (US or DS) and distance from the release point was recorded. A similar resurvey approach was followed for Thorsgill Beck (NE, IF, 2018). For each recaptured crayfish the daily dispersal rate was computed by dividing total distance moved by the number of days between release and recapture dates.

3.2.2.6 Consistency in behavioural traits

At Thorsgill Beck (NE, IF sites) in 2018, for each recaptured crayfish a second set of tests of behavioural traits was performed as described above. This allowed the researcher to test if individuals exhibit repeatable measures for activity, distance moved, exploration, climbing and boldness, and whether any relationship between these behavioural measures also persists over time, indicative of a behavioural syndrome (Cote, Fogarty, *et al.*, 2010).

3.2.2.7 Measuring population density and habitat characteristics

During the resurvey for tagged crayfish in both streams, fine-scale physical characteristics (water depth, wetted width, water velocity and refuge availability) were recorded within the reaches of each study site. The whole study reach (IF and NE sites combined for Thorsgill) was divided into 200 sections, each 5-m long, and water depth, wetted width and bottom substrates were recorded for each of the subsection. Water depth was recorded at 25%, 50% and 75% width positions of the channel across transects at the downstream end, middle and upstream end of each subsection. Three measurements of wetted widths at the downstream end, middle and upstream end of each of the subsection were recorded. Refuge availability is a crucial habitat factor for crayfish (Bubb *et al.*, 2009) and in many upland streams is mostly provided by large unembedded cobbles and boulders (Bubb *et al.*, 2006a). In each 5-m section an index of availability by area of refuges was determined by measuring the size of the unembedded in-stream rocks of ≥250 cm^2 (minimum substrate area required for the smallest crayfish used in this study, 25 mm CL; Streissl and Hödl, 2002) which offer actual or potential refuge to signal crayfish. Refuge availability (as cm^2 m^{-2}) was determined by dividing the total area of all rocks measured by wetted area of the section. Crayfish density for every section was determined by dividing the total number of crayfish captured by wetted area of the section. However, there was no significant variation between pre-survey and resurvey crayfish density results (Welch *t*-test, all $P > 0.05$) and therefore population density data reported earlier were those calculated from the resurvey for tagged crayfish because of a greater number of observations.

3.2.3 Data analysis

3.2.3.1 Behavioural correlations, consistencies and threat response

The repeatability of behavioural traits over time in recaptured crayfish (activity, distance moved, exploration, climbing and boldness score in NE and IF) was determined by calculating intraclass correlation coefficients (ICC; Lessells and Boag, 1987) using the package 'psych' (Revelle, 2018) in R. As there was no 'after' measurement in Westholme Beck it was not possible to analyse consistency in behaviours for FE. To test for evidence of behavioural syndrome between various behavioural measures, Spearman's correlations were also performed. Differences in crayfish boldness types, recorded during the threat response test (i.e. bold or shy), between the three crayfish sites (N = 310; FE 130, NE 90, IF 90) were analysed using a Fisher's exact test for a 2×3 table (Fisher, 1922).

3.2.3.2 Principal component analysis (PCA)

As the studied behavioural traits were correlated, a Principal Component Analysis (PCA) with varimax rotation (Quinn and Keough, 2002) was performed for each site to define possible personality trait dimensions (Cote, Fogarty, *et al.*, 2010) using the R package 'psych'. Two key PCA factors were identified for further analyses based on the scree plots and a broken-stick model (Jackson, 1993). As sample size was small (n = 25 – 41 for each site) behaviours with a loading of >0.60 were considered to contribute to the meaning of a component (Budaev, 2010).

3.2.3.3 Factors affecting dispersal in streams

In order to determine if there is any effect of population density on dispersal, the crayfish density measured at the subsection where an individual tagged crayfish was recaptured (recapture section) was compared with the mean density of crayfish in all the subsections traversed (sections crossed) by that particular crayfish during dispersal. In streams, crayfish adopt ephemeral home ranges, spending several days at one locality with daytime refuges, emerging to forage at night, before moving to a new locality (Robinson *et al.*, 2000; Bubb *et al.*, 2006b). Daytime refuge habitat for signal crayfish has specific characteristics (Bubb *et al.*, 2006b) and strong competition for refuges can be evident (Gherardi and Cioni,

2004; Bubb *et al.*, 2009) making daytime refuge use unlikely to be a random process. Dispersal in crayfish in permanent streams, particularly in an upstream direction, is therefore a stepwise process, by comparison to, for example, birds dispersing when they fledge. A similar analytical approach was also used for the determination of relationships with water depth and refuge availability.

Generalised Linear Models (GLMs) were used for each crayfish site (FE, NE, IF) to determine the drivers of dispersal, using type III *F*-tests with the 'car' package in R (Fox and Weisberg, 2011). A global model was developed for each crayfish site, including all the behavioural trait dimensions (as PCA scores), body mass, population density and habitat characteristics (water depth and refuge availability), missing claw/leg (Yes / No) and sex (Male / Female) as predictor variables with dispersal rate as the response variable. The global model was subset to select the final 'reduced / simplified' model, based on the AICc value (lowest) and model weight, for each crayfish site using the 'MuMIn' package in R (Bartoń, 2019). However, all the subset models with ΔAICc values of less than 2 were recorded as they can effectively predict field behaviour of animals (Burnham and Anderson, 2002) and are equal in theory, a model averaging procedure was employed to select the final model including all important variables in R (Bartoń, 2019) for analysis. Effects of sex or missing claw/leg on dispersal rate for each site were tested separately using Generalised Linear Models (GLMs) in R (package 'lme4'; Bates *et al.*, 2015) with sex and missing claw/leg as fixed effects.

To determine if dispersal is biased towards any specific direction (upstream or downstream or none) a GLM was used for each crayfish site, with dispersal rate as the response variable and dispersal directions as predictors. As the data were overdispersed (Kleiber and Zeileis, 2008) a negative binomial regression model was employed for the analysis. There was a small variation in number of days crayfish remained at liberty and so this was added as an 'offset' to the GLM models, thus ensuring dispersal was estimated on a standardised scale (as m day^{-1}). Influence of sex or missing leg/claw on dispersal direction was analysed by using Fisher's exact test.

Before analysis, data for body mass, behavioural traits, population density and habitat characteristics were divided by the largest value that was measured for the sites which resulted in a proportion for each variable and were normalized to values between zero and 1 (Edwards *et al.*, 2018). However, since some variables contained negative values (e.g. habitat characteristics), they were shifted to positive by adding all values with the absolute of the most negative (minimum value) so that the most negative one became zero (Teknomo, 2015). All analyses were carried out in R (R Core Team, 2017) considering an α significance level of 0.05.

3.3 Results

In Westholme (FE), a total of 2659 crayfish were captured during the September recapture survey (female: 1225, male: 1175, unidentified: 259) of which 41 were marked crayfish (recapture rate: 31.5%). In Thorsgill, 1424 crayfish were recaptured (female: 628, male: 749, unidentified: 47) of which 57 were marked, including 25 individuals from the IF and 32 individuals from the NE sites (recapture rate: IF, 27.8%; NE, 35.6%). There was no significant variation in crayfish sex ratio between the marked and released sample and recaptured samples for FE, NE and IF (chi-square tests, all $P > 0.05$); although a lower proportion of female were recaptured in FE (Table 3.2).

3.3.1 Behavioural consistency and threat response

Among the individuals from the NE and IF samples that were subjected to repeated behavioural tests, there was highly significant repeatability for all behavioural measures over time in both NE and IF sites (all $P \leq 0.022$) with high repeatability values in the majority of the cases (ICC, $R = 0.39 – 0.94$; Table 3.3). The proportion of crayfish classified as bold or shy based on the response to the startle test differed across the sites of the two streams (Fisher exact test, $P < 0.001$). In the FE population of Westholme 42% of behaviourally assayed crayfish were bold, whereas a greater proportion of bold individuals were recorded in the NE (52.2%) and IF (72.2%) sites of Thorsgill Beck (Figure 3.3).

TABLE 3.3 Behavioural consistency and repeatability of behaviours in signal crayfish, measured over time in Thorsgill Beck, determined by intraclass correlation coefficients.

Population and behaviour	Repeatability			
	ICC	*df*	*F*-value	*p*-value
NE				
Activity	0.79	31, 32	8.4	<0.001
Distance moved	0.84	31, 32	11	<0.001
Exploration	0.89	31, 32	18	<0.001
Climbing	0.46	31, 32	2.7	0.003
Boldness	0.69	31, 32	5.5	<0.001
IF				
Activity	0.85	24, 25	12	<0.001
Distance moved	0.94	24, 25	33	<0.001
Exploration	0.90	24, 25	19	<0.001
Climbing	0.39	24, 25	2.3	0.022
Boldness	0.56	24, 25	3.6	0.002

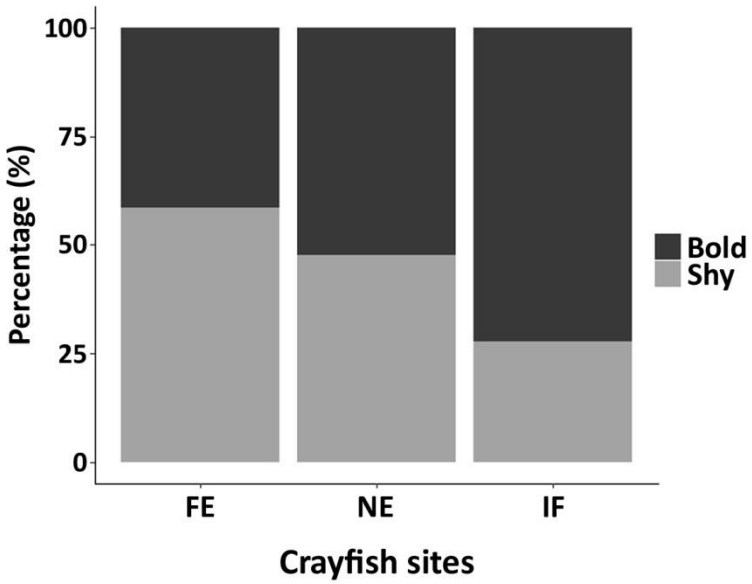


FIGURE 3.3 Proportion of signal crayfish classified as bold or shy based on the response to the startle test at three sites (FE, fully-invaded; NE, newly-invaded; and IF, invasion front) of two streams (Westholme and Thorsgill becks).

3.3.2 Correlations between crayfish behaviours and PCA analyses

The majority of the behaviours measured in the assays were significantly correlated to each other, although the direction of correlation did vary across sites (Table 3.4). Activity and distance moved in the test arena were positively correlated in all three sites (all $P \leq 0.001$). Climbing duration was also significantly positively correlated with activity and distance moved in FE but not in NE or IF (Table 3.4). Exploration was negatively correlated with activity in all sites whereas it was positively correlated with climbing duration in the NE and IF sites of Thorsgill. There was a significant negative correlation between distance moved and exploration in NE. Boldness score was significantly correlated, either negatively (with activity and distance moved) or positively (with climbing and exploration), in all crayfish sites except for climbing in FE (Table 3.4).

TABLE 3.4 Spearman's rank correlations, based on first behavioural test, among the behavioural traits at fully-established (FE), newly-established (NE) and invasion front (IF) sites of signal crayfish of Westholme and Thorsgill becks.

Groups	Distance	Climbing	Exploration	Boldness
FE				
Activity	0.59, *p*<**0.001**	0.41, *p*=**0.007**	–0.34, *p*=**0.032**	–0.60, *p*<**0.001**
Distance		0.46, *p*=**0.002**	–0.09, *p*=0.571	–0.40, *p*=**0.010**
Climbing			–0.07, *p*=0.686	–0.15, *p*=0.349
Exploration				0.58, *p*<**0.001**
NE				
Activity	0.66, *p*<**0.001**	0.15, *p*=0.409	–0.35, *p*=**0.048**	–0.37, *p*=**0.039**
Distance		–0.10, *p*=0.601	–0.44, *p*=**0.011**	–0.35, *p*=**0.050**
Climbing			0.36, *p*=**0.041**	0.37, *p*=**0.037**
Exploration				0.61, *p*<**0.001**
IF				
Activity	0.62, *p*=**0.001**	0.03, *p*=0.885	–0.48, *p*=**0.015**	–0.44, *p*=**0.029**
Distance		0.08, *p*=0.692	–0.28, *p*=0.176	–0.45, *p*=**0.023**
Climbing			0.41, *p*=**0.044**	0.48, *p*=**0.015**
Exploration				0.72, *p*<**0.001**

PCA analyses revealed that two factors (axes) explained 75% (FE), 79% (NE) and 80% (IF) of the variances (Table 3.5, Figure 3.4). Activity and distance moved were on the same PCA axis (PC1 for FE and NE and PC2 for IF) whereas boldness and exploration were on the opposite axis to activity/distance. Component loading for climbing varied among sites; in FE the loading was 0.80 on PC1 (activity–distance), in IF it was 0.85 on PC1 (boldness-exploration) and in NE it was 0.83 on PC2 (with boldness–exploration) (Table 3.5).

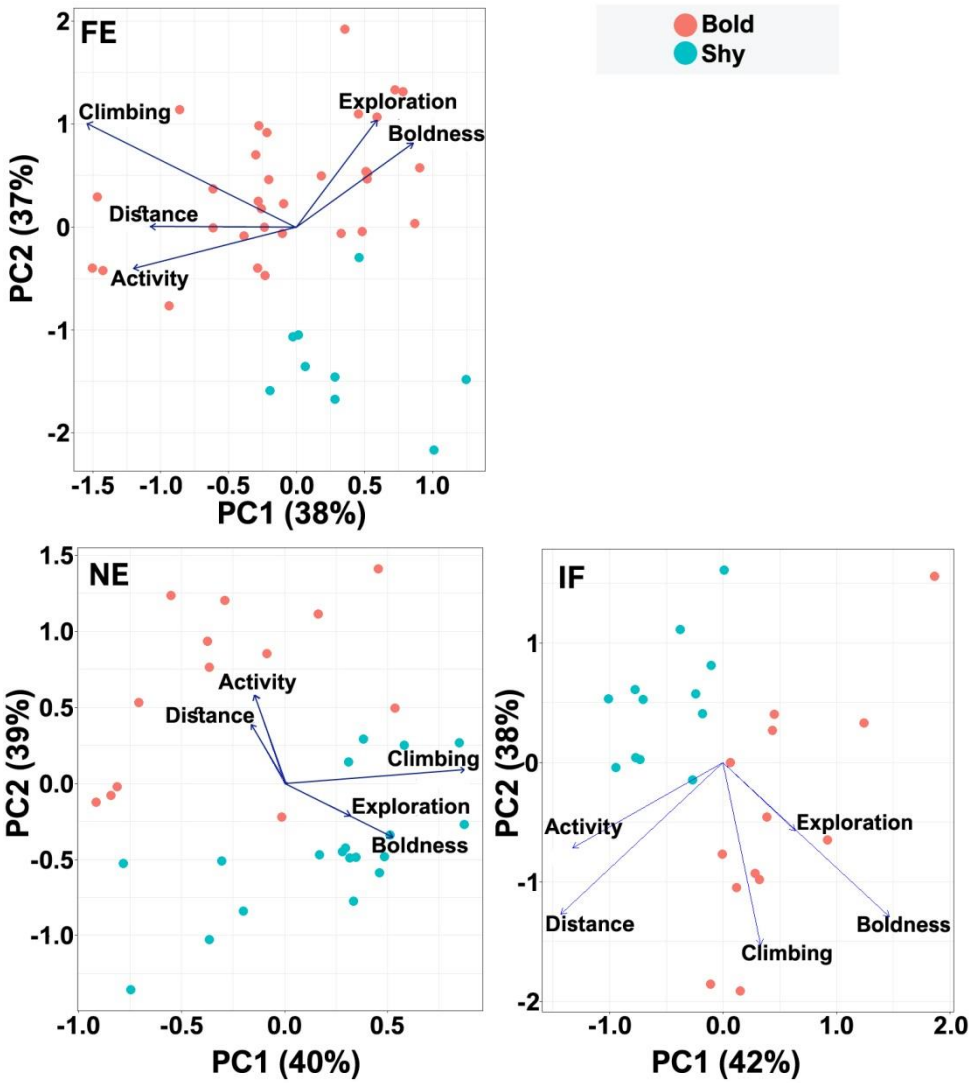


FIGURE 3.4 Principal component analyses showing dimensions of signal crayfish behavioural traits at different sites (FE, full-established; IF, invasion front; NE, newly-established).

TABLE 3.5 Component loadings of crayfish behaviours, obtained through principal component analysis with a varimax rotation. Boldface indicates the highest component loadings for each behaviour.

Groups and behaviours	Principal components	
FE	Activity–Distance–Climbing	Boldness–Exploration
Exploration	0.08	**0.85**
Activity	**0.68**	–0.54
Distance moved	**0.80**	–0.35
Boldness	–0.37	**0.83**
Climbing	**0.80**	0.15
Variance explained (%)	38	37
Total variance (%)	75	
NE	Activity–Distance	Boldness–Exploration–Climbing
Exploration	–0.46	**0.78**
Activity	**0.93**	–0.05
Distance moved	**0.89**	–0.18
Boldness	–0.31	**0.79**
Climbing	0.19	**0.83**
Variance explained (%)	40	39
Total variance (%)	79	
IF	Boldness–Exploration–Climbing	Activity–Distance
Exploration	**0.81**	–0.36
Activity	–0.23	**0.86**
Distance moved	0.00	**0.90**
Boldness	**0.82**	–0.35
Climbing	**0.85**	0.31
Variance explained (%)	42	38
Total variance (%)	80	

3.3.3 Dispersal in streams

At the FE site, one crayfish (2.4% of all FE recaptures) did not move from the section where it was released (Figure 3.5). In Thorsgill Beck two IF crayfish (8% of recaptures for that site) and 5 NE crayfish (15.6% of the recaptures for that site) did not disperse during the study (Figure 3.5).

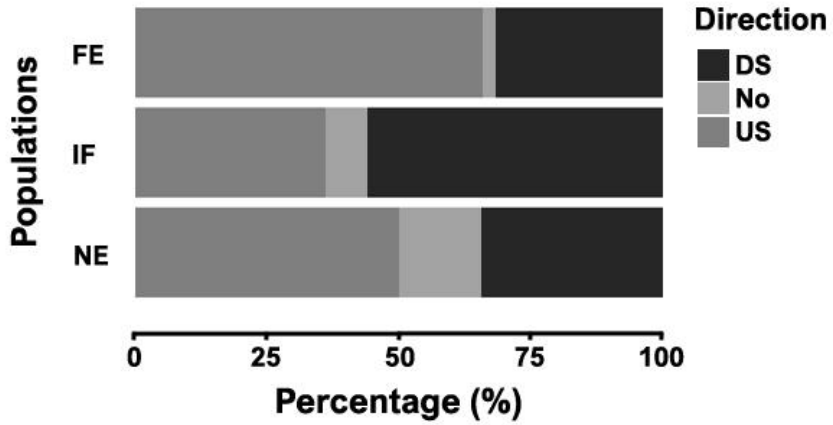


FIGURE 3.5 Proportions of crayfish dispersed towards different directions (DS, downstream; No, did not disperse; US, upstream) at three study sites (FE, fully-established; IF, invasion front; NE, newly-established).

Mean (± SD and range) absolute dispersal distances were 34.1 ± 28.1 m (range: 0 – 125 m), 27.8 ± 28.3 m (0 – 110 m), 34.2 ± 26.4 m (0 – 100 m) in FE, NE and IF sites respectively (Figure 3.6). Mean absolute upstream dispersal distance in IF was 20.6 ± 14.7 m (range: 5 – 40 m). Dispersal direction was mainly upstream in FE (65.9% of the total recaptured crayfish) and NE (50%) but dispersal was mainly downstream in IF (32% upstream). There was no significant difference in dispersal rate between upstream and downstream directions in the FE site (mean ± SD: US, 1.23 ± 1.01 m day^{-1}; DS, 1.22 ± 0.96 m day^{-1}) and the NE site (US, 1.15 ± 1.16 m day^{-1}; DS, 1.26 ± 0.96 m day^{-1}). At the IF site, the upstream dispersal (mean ± SD, 0.71 ± 0.49 m day^{-1}) rate was significantly lower than downstream (1.45 ± 0.76 m day^{-1}) dispersal (GLM, $z = -2.63$, $P = 0.008$; Figure 3.7). No significant effect of crayfish sex or missing claw/leg on dispersal direction was recorded at crayfish sites (Fisher exact test, all $P > 0.05$).

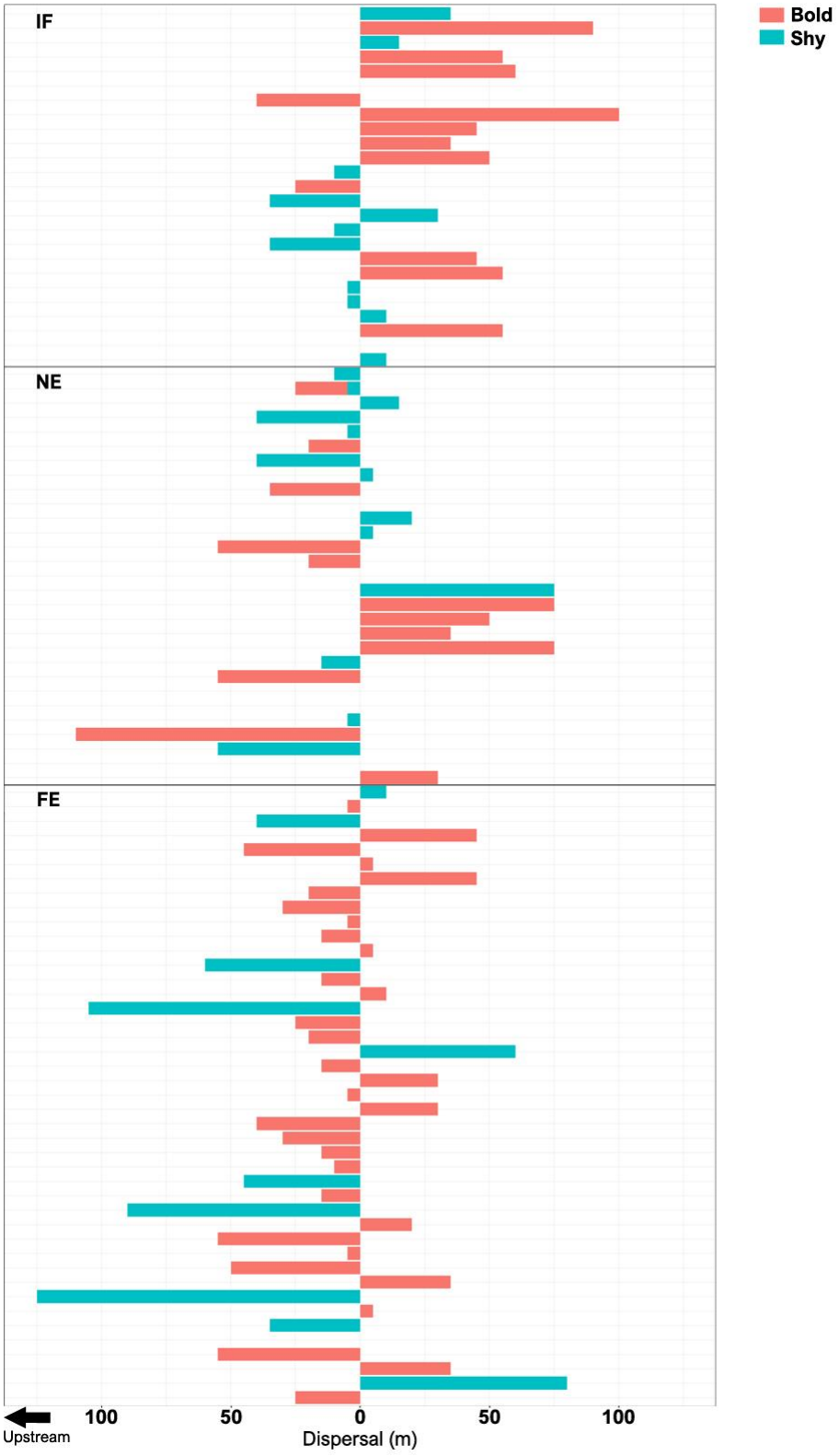


FIGURE 3.6 Absolute dispersal and its directions of signal crayfish in different sites (FE, fully-invaded; IF, invasion front; NE, newly-invaded).

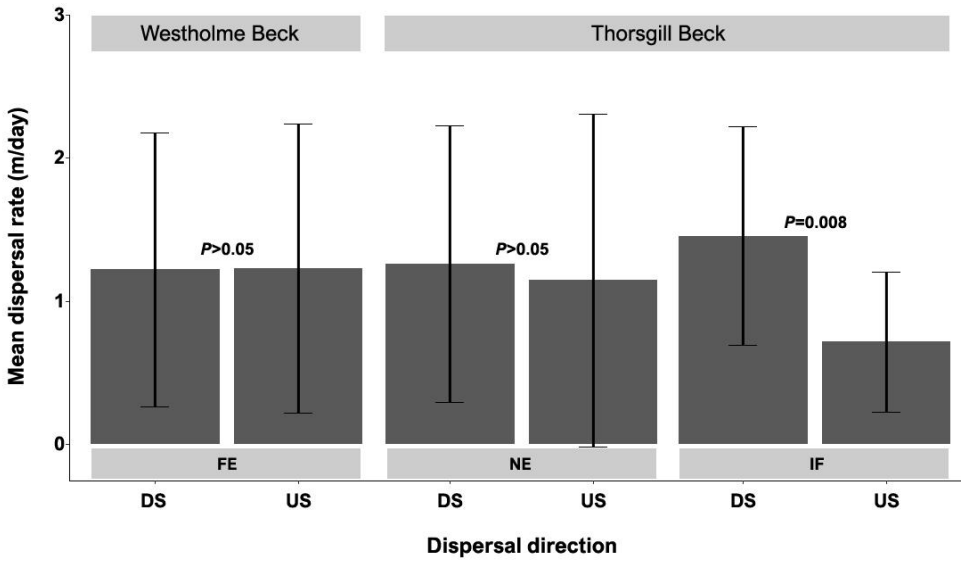


FIGURE 3.7 Mean dispersal rate (± SE, m day^{-1}) at three crayfish sites of Westhholme and Thorsgill becks. DS, downstream; US, upstream; FE, fully-established; NE, newly-established; IF, invasion front.

3.3.4 Factors affecting dispersal

For the FE site, Westholme, the final model of factors affecting dispersal included the exploration–boldness axis and refuge availability (AICc, –31.7; weight, 0.13; see Table 3.6 for details). For NE, the final models included the boldness–exploration–climbing axis, population density and refuge availability (AICc, –7.3; weight 0.08) and both behavioural axes and water depth were retained for the IF model (AICc, –2.6, weight, 0.27) respectively (Table 3.6).

At the FE site, dispersal rate was significantly negatively affected by the boldness–exploration axis ($P < 0.001$) and positively affected by refuge availability ($P = 0.006$; Table 3.7, Figure 3.8). Therefore, more bold and exploratory individuals at the FE site tended to exhibit low dispersal rates but they moved toward sections with higher refuge availability. At the NE site, boldness–exploration–climbing axis and refuge availability had positive significant effects on dispersal rate. More bold and exploratory individuals

dispersed at a higher rate and, similar to FE, also toward sections of river with more refuge availability and less crayfish density.

Table 3.6 Summary of the subset models yielded from global model for each crayfish site, based on model statistics. All the models with ΔAICc value <2 are included here (Burnham and Anderson, 2002) and the top model (also the final model based on model averaging technique) was used for further analysis to reveal the relationships with crayfish dispersal rate.

Group	Model structure	*df*	logLink	AICc	ΔAICc	Weight
FE	PC2+Refuge	4	20.4	–31.7	0	0.125
	PC2+Refuge+Mass	5	21.65	–31.6	0.12	0.118
	PC2+Refuge+Claw	5	21.2	–30.7	1.01	0.076
	PC2+Refuge+Depth	5	21.1	–30.4	1.28	0.066
	PC1+PC2+Refuge	5	20.9	–30.1	1.57	0.057
	PC2+Claw+Mass+Refuge	6	22.2	–29.9	1.83	0.050
	PC2+Claw+Depth+Refuge	6	22.1	–29.8	1.88	0.049
NE	PC2+Density+Refuge	5	9.8	–7.3	0	0.083
	PC2+Refuge	4	8.3	–7.1	0.17	0.077
	PC2	3	6.9	–6.9	0.38	0.069
	PC2+Depth+Refuge	5	9.5	–6.8	0.5	0.065
	PC2+Claw+Depth+Refuge	6	10.8	–6.3	1.01	0.050
	PC1+PC2+Density+Refuge	6	10.8	–6.2	1.08	0.049
	PC2+Claw+Density+Refuge	6	10.7	–6.1	1.16	0.047
	PC2+Depth	4	7.77	–6.1	1.21	0.046
	PC2+Claw+Refuge	5	9.14	–6.0	1.31	0.043
	PC2+Density+Depth+Refuge	6	10.5	–5.7	1.55	0.038
	PC1+PC2+Refuge	5	8.9	–5.5	1.82	0.034
	PC1+PC2+Depth+Refuge	6	10.3	–5.3	1.97	0.031
IF	PC1+PC2+Depth	5	7.9	–2.6	0	0.265
	PC1+PC2+Depth+Refuge	6	9.1	–1.5	1.14	0.150
	PC2+Depth	4	5.4	–0.9	1.73	0.111

At the IF site, there was a significant positive impact of boldness–exploration–climbing axes and an additional negative impact of activity–distance on dispersal rate (Table 3.7, Figure 3.8). Therefore, bold and exploratory individuals and those that performed more climbing dispersed at a greater rate and the opposite trend was true for more active individuals during the behavioural assays (Figure 3.8). At the IF site, crayfish which dispersed at a greater rate also moved toward sections with relatively low water depth. GLM results confirmed no significant impacts of crayfish sex and missing claws/legs on dispersal rate for each site (all $P > 0.05$). Dispersal direction was not significantly influenced by the behavioural traits except for the activity–distance moved axis at the IF site, where individuals that did not disperse had significantly higher activity-distance moved scores than those which dispersed downstream (Figure 3.9).

TABLE 3.7 Dispersal rate in relation to personality traits, physical characteristics, population density and refuge availability. Factors with blank cells were not included in the final model.

Factors	FE	NE	IF
Density	–	F=2.7; P=0.109	–
Refuge	F=8.6; P=**0.006**	F=4.9; P=**0.036**	–
Depth	–	–	F=6.8; P=**0.017**
Activity–Distance	–	–	F=15.8; P<**0.001**
Exploration–Boldness	F=24.8; P<**0.001**	–	
Exploration-Boldness–Climbing		F=8.4, P=**0.007**	F=4.9; P=**0.045**

Relative refuge availablity was similar in all three study sites (P = 0.238; Figure 3.10). However, as expected, signal crayfish density differed significantly among sites (ANOVA: F = 94.8, P < 0.001; Figure 3.10). Post-hoc test confirmed significant differences among all combinations (IF vs. NE, P = 0.032; rest, P < 0.001). Crayfish density was positively related to refuge availability at all sites; FE (R^2 = 0.88), NE (R^2 = 0.80) and IF (R^2 = 0.60).

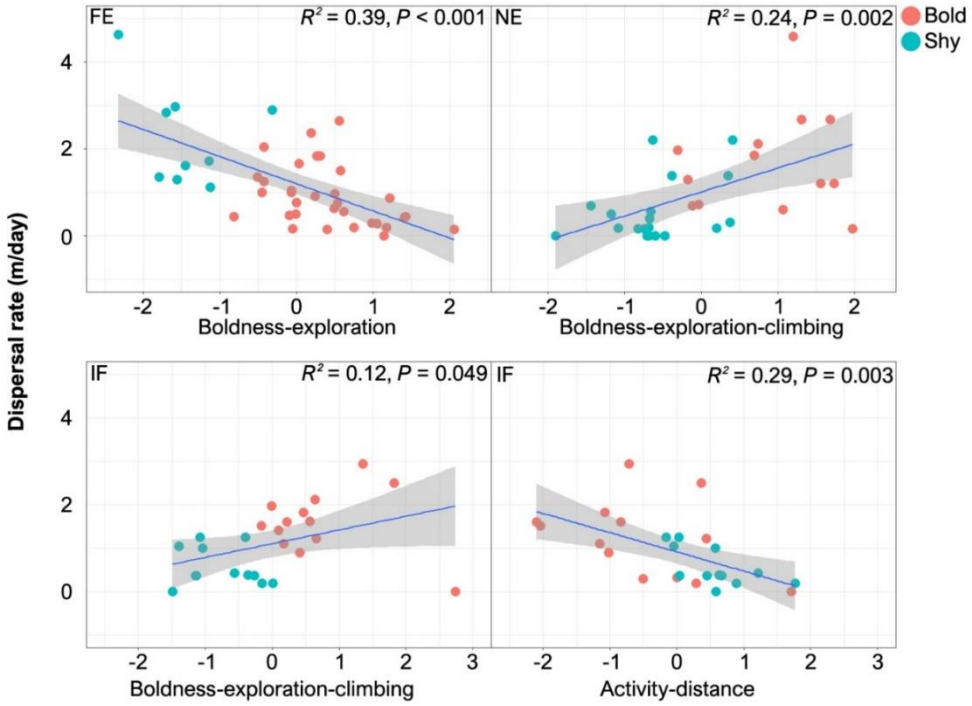


FIGURE 3.8 Linear regressions showing relationships between behavioural traits and dispersal rate at different crayfish sites. FE, fully-established; NE, newly-established; IF, invasion front.

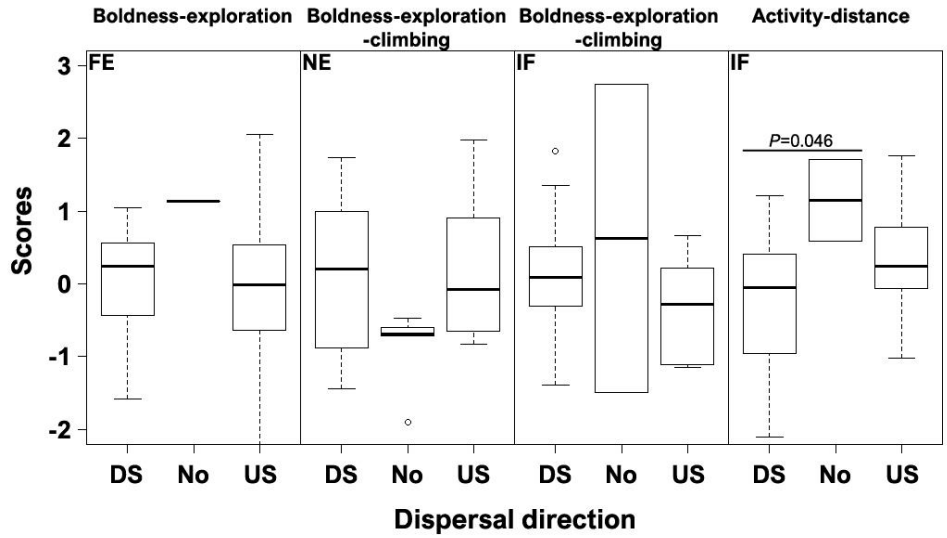


FIGURE 3.9 Boxplots showing relationships between different behavioural axes and dispersal directions (DS, downstream; No, did not disperse; US, upstream) in fully-established (FE), newly-established (NE) and invasion front (IF). Midline within the box is the median; upper and lower limits of the box represent the third and first quartile (75th and 25th percentile) respectively. Significant differences are illustrated and *P* value provided.

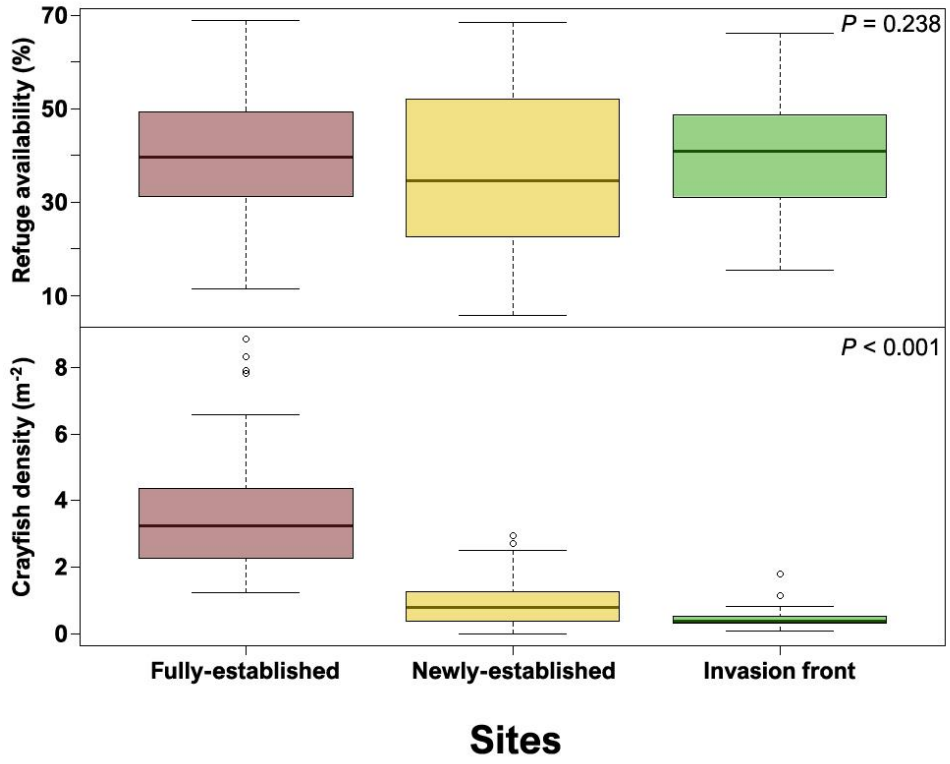


FIGURE 3.10 Boxplots showing refuge availability (above) and crayfish density (below) at three study sites. Midline within the box is the median; upper and lower limits of the box represent the third and first quartile (75th and 25th percentile) respectively. Significant differences are illustrated and *P* value provided.

3.4 Discussion

All hypotheses proposed in section 3.1 were supported by the data obtained. Signal crayfish demonstrated behavioural syndromes, stable over periods of approximately 1 month. The study provides evidence that dispersal of invading signal crayfish is driven by both individual personality and habitat characteristics. It also suggests that the same personality trait can play a varying role in species dispersal, depending on the population status; in otherwords it is context-dependent.

For both NE and IF sites, signal crayfish exhibited very strong individual consistency for focal behaviours over time. High repeatability was

found in most of the behaviours at all sites and combinations of these significantly correlated behavioural traits indicated the existence of behavioural syndromes. However, the structures of behavioural syndromes varied across sites. At the FE site, positive relationships were observed among activity, distance moved and climbing but these three traits were negatively related to boldness and exploration. It might be assumed that more active individuals would move over a longer distance compared to less active conspecifics and, consequently, appear to be more exploratory and bold, as recorded for mosquitofish (Cote, Fogarty, *et al.*, 2010). However, this was not the case in the present study where more active individuals were superficial explorers and tended to explore a smaller area. This may be due to more active, shy crayfish searching for shelters in which to hide (Vainikka *et al.*, 2011), that involved travelling over longer distances because of repeated exploration, mostly along the edges of the experimental arena. Such behaviour needs to be appreciated in the context that crayfish are nocturnal and that carrying out behavioural assays by day, may generate, to a greater or lesser degree, behaviours linked to searching for refuges.

This study provides evidence that personality traits can exhibit different effects relevant to population ecology that are dependent upon context. In this study, the different contexts comprised sites that represented different phases of invasion. Varying relationships were found between boldness-exploration and dispersal rate including a negative relationship in FE but a positive effect in NE and IF. Both positive and negative impacts of boldness-exploration on dispersal distance have been reported, but not for the same species and within close geographical distance. Fraser *et al.* (2001) demonstrated a positive relationship between boldness and distance moved in the field in a fish species (*Rivulus hartii*) and a similar positive relationship was observed between aggressiveness (often linked to boldness) and dispersal in the bird species *Parus major* (Dingemanse *et al.*, 2003). In contrast, Cote, Fogarty, *et al.* (2010) found a negative relationship between boldness and dispersal distance in mosquitofish (*G. affinis*). This study provides the first evidence that the same behavioural traits can yield different dispersal outcomes for the same

species. In this study the crayfish density, and so competition for food and shelter, was high at the FE site compared to NE and IF and thus, shy individuals were likely outcompeted by bolder counterparts and therefore dispersed over relatively longer distances, compared to bolder individuals at the FE site. At the NE and IF sites, bolder individuals dispersed more. Although competition for shelter is likely to be less in these newly colonised areas a high dispersal rate may not be expected for shy crayfish, as observed in this study, because they spend more time in shelters than bold individuals, even in the absence of predation risk (Vainikka *et al.*, 2011). On the other hand, bold crayfish are expected to disperse well into new areas (Pintor *et al.*, 2008).

Interestingly, the effect of climbing in the behavioural syndromes as determined by the PCA analyses, a behavioural trait that is not a key focus in most behavioural studies, varied across crayfish sites. Climbing behaviour was aligned with activity–distance moved axis in FE but joins the boldness-exploration axis in the other two sites. This supports the view that the structure of behavioural syndromes might vary with context and population density, and that competition and predation pressure may play a key role in this regard (Bell and Sih, 2007; Pintor *et al.*, 2008; Smith and Blumstein, 2008). It may be speculated that this shifting is due to the adaptive plasticity of individuals across sites; to survive in an environment with high competition (i.e. FE) shy crayfish exhibit higher levels of behavioural plasticity like being more active and climbing to cope with the higher level of competition. In experiments with pairs of signal crayfish, increased climbing activity was exhibited by subordinate (putatively shy) individuals as an escape mechanism from aggressive dominant individuals (Rice *et al.*, 2012) which somewhat reflects the adaptive plasticity of individuals. On the other hand, in NE and IF, bold crayfish may have performed more climbing as a part of regular dispersal to exploit novel resources. However, it is common in nature that within a species an individual's response may differ depending on context (Dowding *et al.*, 2010; Legagneux and Ducatez, 2013). Individuals living in an environment with more modification (e.g. wildlife in urban areas, when compared to counterparts in nonurban areas) often exhibit more behavioural flexibility,

sometimes innovative behaviours (Lefebvre, 1995; Bouchard *et al.*, 2007; Wong and Candolin, 2015).

Positive relationships between climbing (along with boldness-exploration) and dispersal rate in both NE and IF were recorded which may indicate that climbing behaviour has a significant role in expanding population range through dispersal. This result is of particular interest in the case of crayfish because previous studies have showed that in-stream obstacles, both artificial and natural and either large (Light, 2003; Kerby *et al.*, 2005) or small (Light, 2003; Bubb *et al.*, 2006a, 2009; Gil-Sánchez and Alba-Tercedor, 2006) can limit crayfish distribution. Thus, it may be assumed that individuals with higher climbing ability and/or persistence have greater chances to pass a barrier, especially if it is a small one.

Although personality traits significantly affected dispersal rates in signal crayfish in this study, environmental factors were also important determinants, again with effects that were context dependent. Greater availability of refuges was related to a higher dispersal rate in FE and NE sites, but not at the IF site. Previous studies have suggested that crayfish distribution is influenced by shelter availability (Lodge and Hill, 1994; Streissl and Hödl, 2002). Competition for refuges will be lower at the IF because of the low density of crayfish there but it would be higher in localities with high crayfish density, as is the case for NE and FE. Water depth was significantly negatively related to dispersal rate in the IF (site crayfish tended to disperse from deep water sections to shallow areas). However, the tendency to move to a shallow area, primarily by crayfish of <35 mm CL, is often related to reduce predation risk from fishes in deeper sections (Englund and Krupa, 2000; Guan, 2000). This might be explained by the presence of brown trout in the study streams, a common predator of crayfish.

No significant effect of crayfish sex, missing claw/leg or body mass on their dispersal was recorded in this study. It is quite unlikely that missing claws/legs did not affect dispersal. However, the maximum dispersal distance recorded in this study was only 125 m and dispersal rate was quite slow (<5 m day^{-1}) and thus it is possible that crayfish with missing

claw/leg(s) also managed to disperse over similar distances. Autotomy of limbs is common in decapods including crayfish (Wood and Wood, 1932; Holdich, 2002) during predation attempts and during fighting and although it impacts growth (Holdich, 2002), evidently it seems to influence dispersal less than behavioural syndrome. Stream width may play an important role in crayfish dispersal because signal crayfish have been reported to move 341 m distance in just two days in a 30-m wide river (Bubb *et al.*, 2006b) which is much wider than the streams in the present study. Although dispersal can be sex-biased (e.g. in *Parus major*; Duckworth and Badyaev, 2007) this may not be the case in aquatic fauna (in *R. hartii*, Fraser *et al.*, 2001) as recorded in this study.

The direction of dispersal has never been considered in previous studies of personality-dependent dispersal. While direction of dispersal may be less important to consider for terrestrial or avian fauna as they usually expand ranges in any direction, for a running aquatic ecosystem, direction of dispersal is an important aspect to quantify. Although downstream dispersal rate was significantly higher than upstream dispersal at the IF, no significant influence of behavioural traits was found in determining direction of dispersal. Downstream dispersal at a higher rate than upstream dispersal of signal crayfish has also been reported in other streams (Bubb *et al.*, 2005; Weinländer and Füreder, 2009) which may have relevance to the water flow but this issue requires further investigations. Interestingly, more bold individuals moved downstream towards the source population but it was also the bold individuals who made the longest dispersal towards upstream. This reflects a stronger tendency of bold individuals to disperse in an invaded habitat. Moreover, it should not be interpretedthat shy individuals are leading the invasion as more shy individuals dispersed upstream. This is because these crayfish were captured and released at different locations within a 75-m long stretch and movement towards upstream does not necessarily mean that they are leading the invasion, especially when no shy crayfish dispersed more than 35 m. However, short upstream dispersal in IF indicates a slow range expansion towards upstream during the time of the year when signal crayfish remain most active (Bubb *et al.*, 2002, 2004). There is a reproductive disadvantage to

animals, including crayfish, through dispersing to such an extent that the encounter rate with potential mates falls to suboptimal levels, reducing fitness (Allee effect; Greene, 2008). Therefore, at the IF there is expected to be a trade-off in dispersal to reduce intraspecific competition and increase growth, while also maximising reproductive output. Determining dispersal direction and range expansion for aquatic taxa may have strong relevance to conservation or management goals. For example, this may help us in selecting an appropriate place for any intended conservation action (e.g. deployment of a barrier, trapping or control methods) to control or limit invasive population. Potential management strategies using the study findings may involve alteration of refuge availability in invaded habitat and use of structures (e.g. in-stream barriers) that can affect crayfish behaviours (e.g. climbing). More specific examples of invasive crayfish management using the study results are presented in General Discussion chapter (section 5.3, p. 188).

3.5 Conclusions

This study provides evidences that crayfish behaviours that are associated with dispersal exhibit consistency over time and form behavioural syndromes. But the form of these syndromes and the impact of behavioural type (boldness-shyness, exploration) on dispersal is very context dependent. Additional environmental factors also influence dispersal and these too are context dependent. This confirms that the signal crayfish are filtered by behavioural traits and the environment to fractionate their population by personality along the invasion gradient (Figure 3.11). The bold and exploratory individuals with more climbing ability appear to be more efficient in dispersing further at the invasion front and newly-established sites whereas this was not the case at fully-established site. Therefore, understanding the progress of invasive species, especially those are in linear aquatic systems, requires a combined understanding of the personality traits and variation within the species or population and local habitat complexity. Biological invasion causes substantial economic and ecological damage worldwide and thus management is a global concern

(Pimentel *et al.*, 2001; Luque *et al.*, 2014). Existing management plans for non-native species usually do not consider variation in personality traits within populations, let alone the context dependency of personality effects on dispersal. Such gaps in our knowledge can decrease the efficiency of management plans by leading to misdirected efforts (Juette *et al.*, 2014). The findings of this study may contribute significant knowledge to management strategies of non-native crayfish (see section 5.3 in General Discussion for specific examples, p. 188), but also highlight the importance of understanding the context-dependency of personality effects on the dispersal of invasive species.

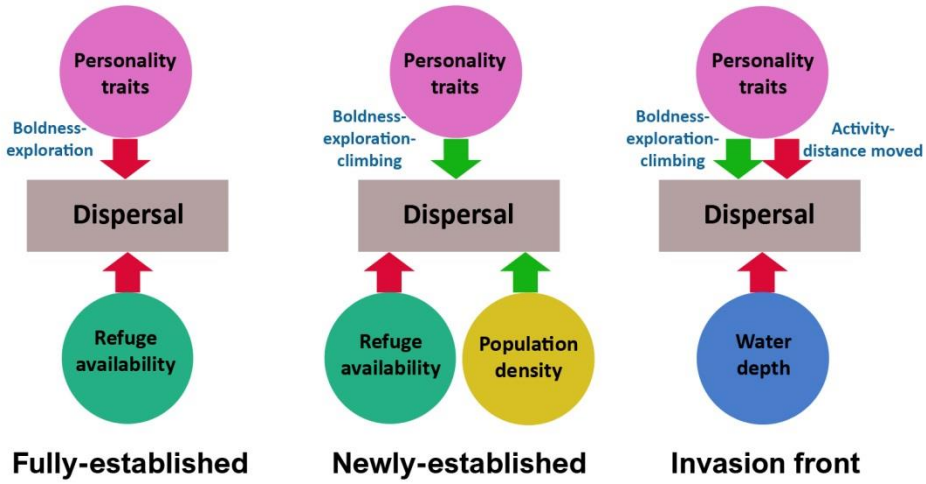
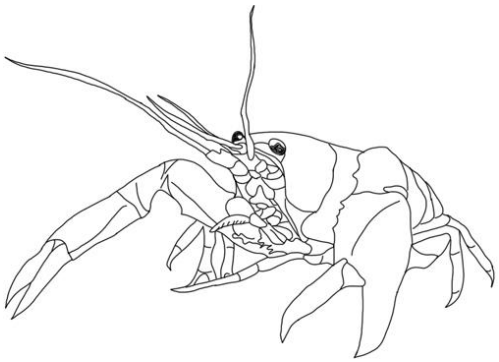


FIGURE 3.11 Conceptual diagram showing the influences (positive, green arrows; negative, red arrows) of crayfish personality and various environmental factors found across different phases of invasion.



Chapter Four

ASSESSING IMPACTS OF SIGNAL CRAYFISH INVASION ON UPLAND STREAM FISH AND INVERTEBRATE COMMUNITIES

Summary

Impacts of invasive signal crayfish *Pacifastacus leniusculus* on native species and ecosystems are widely recognised, but mostly through small-scale studies and controlled laboratory experiments that may not always reflect impacts in nature. Recorded effects of signal crayfish on fish populations have been equivocal. In this study, using the before-after-control-impact and control-impact approaches, the effects of signal crayfish invasion on native fish species, particularly benthic fishes and young-of-year (YoY) salmonids, and macroinvertebrate communities were determined on different spatial and temporal scales through three linked studies (S_1–S_3), in upland streams of the River Tees, northeast England.

In S_1, fish and macroinvertebrate communities of 18 streams were sampled identically in 2011 and 2018. These streams were broadly categorised into two groups, (i) uninvaded (without signal crayfish in both sampling years; n = 7) and (ii) invaded (with crayfish) streams. There were two types of invaded streams, pre-invaded (invaded by signal crayfish before 2011; n = 8) and (iii) newly-invaded (invaded by signal crayfish between 2011 and 2018, n = 3). Despite similar habitat conditions fish and macroinvertebrate communities changed significantly over time in pre-invaded streams and by comparison to uninvaded streams. A decline in the abundance of benthic fish and YoY salmonids was observed in newly-invaded streams. Complete disappearance of bullhead *Cottus gobio* following signal crayfish invasion was recorded in two pre-invaded streams.

In the second study, S_2, within-stream differences in fish and macroinvertebrate communities and abundance in two streams were assessed by comparing sections with (invaded) and without (uninvaded) signal crayfish. Compared to uninvaded sections, both richness and abundance of fish and macroinvertebrate were significantly lower in invaded sections and the overall community also differed significantly.

In S_3, long-term data series (since 1990) of water quality and macroinvertebrates of six streams including both signal crayfish invaded and uninvaded streams were analysed. Water quality showed little change, or an improvement, over time but significant changes in the macroinvertebrate taxonomic richness and community structure occurred following signal crayfish invasion in invaded streams whereas significant changes were also recorded in uninvaded streams but in a different direction. Long-term changes in macroinvertebrate communities in invaded streams tended to be due to be due to declines in more sedentary taxa such as molluscs and cased trichopterans.

Taken together, these three study elements provide strong evidence that widespread and long-term ecological disruption is

occurring because of signal crayfish invasion in upland streams of the Tees catchment. On the evidence gathered, it seems likely that such invasions may lead to a complete disappearance of some benthic fish species, as well as reduced recruitment of salmonids and a shift towards less diverse macroinvertebrate communities, dominated by more mobile, crayfish-resistant taxa.

Keywords: Signal crayfish, non-native, biological invasion, upland rivers, salmonid, benthic fish, macroinvertebrate, conservation

A concise version of this chapter, lead by S Galib and co-authored by J Findlay (see below for contribution) and M Lucas (supervised and conordinated the study) has been submitted as a manuscript in Freshwater Biology and is currently under review. However, the chapter has benefitted from a first round of review comments by the reviewers.

In the first study S_1, Mr. John Findlay (School of Biological and Biomedical Sciences, Durham University, supervised by M.C. Lucas) collected the field data in 2011.

4.1 Introduction

Biological invasion, as stated earlier in Chapter One, is playing a key role in the decline of biodiversity worldwide (Dudgeon *et al.*, 2006; Naeem *et al.*, 2012; Simberloff *et al.*, 2013). Non-native species can impact the invaded ecosystem directly (e.g. predation, competition and displacement of native species) or indirectly (e.g. trophic cascade), resulting in altered structure and functioning of the receiving ecosystem (Bondar *et al.*, 2005; Strayer, 2010; Gutiérrez *et al.*, 2014). Biological invasions may cause irreparable ecological and economic (Gherardi *et al.*, 2011) or even cultural (Lodge *et al.*, 2012) damage. Crayfish are an important group of non-native species and commonly considered ecosystem engineers, partly because of their role in the alteration of detrital processing rates (Creed and Reed, 2004; Carvalho *et al.*, 2016), and by grazing plants (Creed, 1994; Nyström *et al.*, 2001; Matsuzaki *et al.*, 2009) and so are considered keystone consumers (Gherardi *et al.*, 2011; Reynolds, 2011).

As outlined in Chapter Two, non-native crayfish can alter aquatic biota directly and indirectly through complex interactions (Reynolds, 2011; Jackson *et al.*, 2014; Ruokonen *et al.*, 2014). Their effects may be extensive if they grow to a large size or populations become dense (Strayer, 2010; Gherardi *et al.*, 2011). This can result in an alteration of community composition and functioning (Jackson *et al.*, 2014). Other crayfish species, macroinvertebrates, molluscs, benthic fishes, amphibians, and macrophytes are vulnerable to non-native crayfish invasion (Wilson *et al.*, 2004; Gherardi, Mavuti, *et al.*, 2011; Dorn, 2013; Mathers *et al.*, 2016). Reduced growth rates and feeding of native fish species have been reported in habitats with non-native crayfish (Light, 2005). In some cases with fish predators of invasive crayfish, predatory fish growth has increased (Wood *et al.*, 2017). However, where crayfish grow to a large size they can become resistant to gape-size limited predators including many fish species (Gherardi, Aquiloni, *et al.*, 2011).

Although impacts of invasive crayfishes, including signal crayfish *Pacifastacus leniusculus*, on fishes are known, some evidence is contradictory. The abundance of small benthic fishes (e.g. sculpins and loaches) can be lower in river reaches invaded by signal crayfish than without (Guan and Wiles, 1997; Bubb *et al.*, 2009) and similarly for brown trout *Salmo trutta* in headwater streams (Peay *et al.*, 2009). However, these field data were correlative and measured over short time scales. Other studies found no effect of signal crayfish on trout density (Degerman *et al.*, 2007). From studies on egg and alevin predation by crayfish (Edmonds *et al.*, 2011; Findlay *et al.*, 2015), it has been suggested that impacts on salmonids are likely to be most evident in the first year of life reflecting survival from the spawning redds since, in salmonids, subsequent survival is strongly density dependent, and densities may also alter due to migration (Findlay *et al.*, 2015). However, to date, no study confirmed a relationship between the abundance of YoY salmonids and crayfish in the wild. Laboratory experiments have also revealed that signal crayfish can outcompete benthic fish species (bullhead *Cottus gobio*; and stone loach *Barbatula barbatula*) for shelter and significantly increase mortality of benthic fish (Guan and Wiles, 1997). However, small-scale and controlled

laboratory experiments may not be appropriate for predicting the impacts of crayfish in nature (Degerman *et al.*, 2007). In field studies it is difficult to determine factors, including invasive crayfish, responsible for changes in fish populations, without controlling for habitat and year-to-year recruitment variability, and this issue has not been fully addressed (Degerman *et al.*, 2007; Peay *et al.*, 2009).

Knowledge of impacts of crayfish on biodiversity and ecosystem services are important for formulating management strategies (Lodge *et al.*, 2012; Jackson *et al.*, 2014; Moorhouse *et al.*, 2014). Although several studies have examined the community-scale impacts of invasive crayfishes (Stenroth and Nyström, 2003; Jackson *et al.*, 2014; Mathers *et al.*, 2016) most have been short-term and utilised mesocosm experiments. Responses to invasion have mostly been analysed from spatial comparisons (with vs. without invader e.g. Crawford *et al*., 2006, Ercoli *et al.*, 2015). These do not provide information on temporal invasion impacts. Mathers *et al.* (2016), examining long-term impacts of signal crayfish on lotic macroinvertebrate communities, used a paired control (uninvaded) – intervention (invaded) design of study sites to minimise the likelihood of differences in water quality or stream habitat as being causal in observed changes in invertebrate communities at sites invaded by signal crayfish. However, the situation remains unknown in upland habitats because Mathers *et al.* (2016) only considered lowland rivers and therefore, due to dissimilarities between two stream types, it is not possible to predict similar results in upland rivers. There is a need for longer term studies, covering multiple generations of focal species, ideally employing before-after-control-impact (BACI) methodology, to determine the impact of invasive species such as signal crayfish. Due to the potential for reaching 'tipping points' due to biodiversity loss (Dirzo *et al.*, 2014), determining the extent of ecological impact due to species invasion should also measure the response of multiple taxa such as plants, invertebrates and fishes.

In this study the impacts of non-native signal crayfish were measured through three related studies, using BACI and control-impact (CI) study designs, with consideration of habitat and water quality factors, on native fish populations and invertebrate communities in upland UK

streams to determine the effects of crayfish on native communities in invaded streams, compared to uninvaded habitats, on various spatial (invaded vs. uninvaded streams/stretches) and temporal (one, seven and 28 years) scales. It was hypothesised that signal crayfish would negatively affect community components most likely to be susceptible to benthic interactions with crayfish, small benthic fishes, YoY salmonids and less mobile macroinvertebrate taxa. It was also hypothesised that invasion-mediated faunal impacts operate on a timescale reflective of the period taken for signal crayfish colonisation to achieve densities approaching carrying capacity.

4.2 Materials and methods

4.2.1 Study area and approach

Here, three related studies (hereafter S_1–S_3) were employed to reveal medium- to long- term impacts of signal crayfish within and between habitats. In the first study S_1, eighteen streams of the upper to middle River Tees catchment in northeast England were surveyed for fish and macroinvertebrates in 2011 and 2018 (Figures 4.1 & S4.1, Table 4.1). The Tees has an upland limestone geology, with a hydrological regime dominated by rapid surface run-off in response to rainfall, and riffle-pool streams dominated by mobile, larger sediment particles (cobble, boulder). Historically, large parts of the Tees catchment were inhabited by native white-clawed crayfish *Austropotamobius pallipes*. However, several mass mortalities were recorded in the 1980s and the species had declined dramatically by the 1990s and was almost completely replaced in the 2000s by signal crayfish (Holdich *et al.*, 1995; Holdich, Rogers, *et al.*, 1999; Priestley, 2003). Lartington ponds near Barnard Castle are the primary and original source of signal crayfish colonisation in the Tees catchment, where they were released for the restaurant trade in the 1980s (Stebbing *et al.*, 2004).

White-clawed crayfish were not found at any of this study's survey sites in 2011 and 2018. In this study, tributary streams provided

environments that could be sampled quantitatively for crayfish and fish whereas the main river channel could not. Tributary streams also provided sampling units that were relatively independent from one another, since in most of those invaded by signal crayfish, it is likely that signal crayfish used the main River Tees as a conduit for stream colonisation, given the location of the original stocking site (ponds in a tributary of Deepdale Beck, Figure 4.1, Table 4.1) and the known Tees invasion history (M.C. Lucas, pers. comm.).

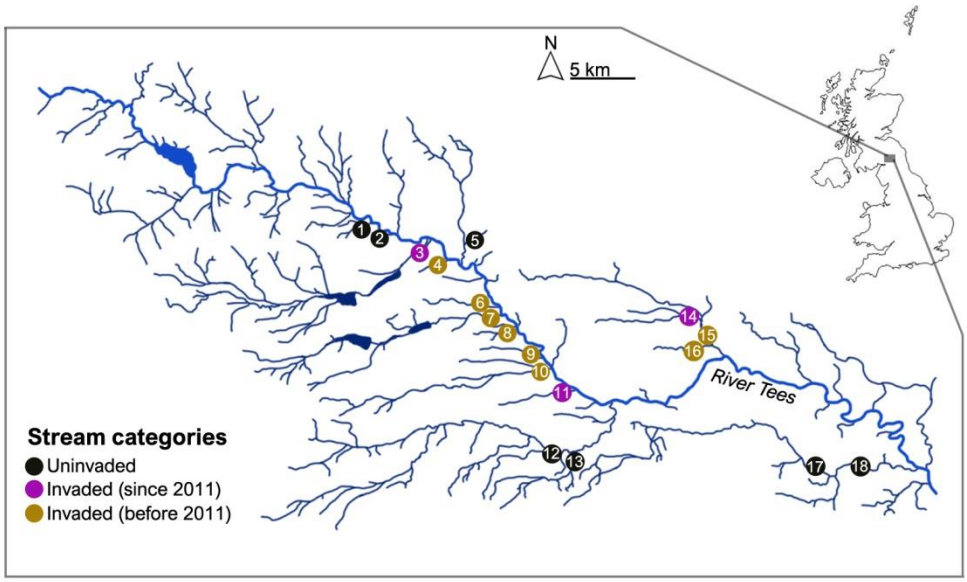


FIGURE 4.1 Map of the study site locations in the River Tees catchment of North East England. 1, Parkend Beck; 2, Unnamed Beck; 3, River Lune; 4, Icaron Beck; 5, Blackton Beck; 6, Wilden Beck; 7, River Balder; 8, Lance Beck; 9, Scur Beck; 10, Deepdale Beck; 11, Thorsgill Beck; 12, River Greta; 13, Gill Beck; 14, Sudburn Beck; 15, Alwent Beck; 16, Westholme Beck; 17, Aldbrough Beck; and 18, Clow Beck. Grid coordinates and stream characteristics are given in Table 4.1.

The surveyed streams were divided into two groups, depending on the status of signal crayfish invasion, comprising (i) uninvaded streams, streams with no signal crayfish over the period 2011–2018; (ii) invaded streams. Invaded streams were further divided into two types, pre-invaded streams, those streams invaded by signal crayfish prior to 2011 and newly-invaded streams, those streams invaded by signal crayfish between 2011 and 2018 (Figure 4.1; Table 4.1).

TABLE 4.1: Location and characteristics of streams sampled in the Tees catchment, classified by invasion condition in 2018. The same sites were surveyed in 2011, providing a BACI sampling methodology. Site numbers refer to those in Figure 4.1.

Site No.	Stream names	Location	Area sampled (m^2)	Width (Mean ± SD; m)		Stream categories[1]
				Channel	Wetted	
1	Parkend Beck	54°37'42"N 2°06'54"W	106.1	4.4 ± 2.1	2.9 ± 1.5	Uninvaded
2	Unnamed Beck	54°37'24"N 2°06'38"W	127	1.5 ± 0.6	1.2 ± 0.4	Uninvaded
3	River Lune	54°37'05"N 2°03'20"W	147.2	10.3 ± 0.4	9.5 ± 1.0	Invaded (since 2011)
4	Icaron Beck	54°36'26"N 2°02'07"W	107.2	3.2 ± 0.8	2.3 ± 0.6	Invaded (before 2011)
5	Blackton Beck	54°37'02"N 02°01'00"W	119.3	3.7 ± 0.3	3.1 ± 0.5	Uninvaded
6	Wilden Beck	54°34'50"N 01°59'44"W	136.8	3.8 ± 0.8	3.3 ± 0.8	Invaded (before 2011)
7	River Balder	54°34'31"N 01°59'13"W	97.8	11.1 ± 0.4	9.8 ± 0.5	Invaded (before 2011)
8	Lance Beck	54°34'11"N 01°57'53"W	130.3	4.5 ± 0.7	3.4 ± 0.5	Invaded (before 2011)
9	Scur Beck	54°33'03"N 01°56'21"W	176.8	6.1 ± 1.9	5.8 ± 2.1	Invaded (before 2011)
10	Deepdale Beck	54°32'42"N 01°55'56"W	121.0	10.7 ± 0.9	5.2 ± 0.5	Invaded (<2011)
11	Thorsgill Beck	54°31'55"N 01°54'19"W	147.8	3.7 ± 0.7	2.4 ± 0.9	Invaded (since 2011)
12	River Greta	54°29'45"N 01°55'46"W	126.4	7.7 ± 0.7	7.2 ± 0.8	Uninvaded
13	Gill Beck	54°29'21"N 01°54'18"W	109.2	5.2 ± 1.1	3.7 ± 1.4	Uninvaded
14	Sudburn Beck	54°34'32"N 01°47'20"W	145.9	4.4 ± 0.5	4.1 ± 0.8	Invaded (since 2011)
15	Alwent Beck	54°33'35"N 01°46'28"W	212.2	7.2 ± 0.5	6.1 ± 1.0	Invaded (before 2011)
16	Westholme Beck	54°33'24"N 01°46'45"W	121	2.7 ± 0.9	2.3 ± 0.6	Invaded (before 2011)
17	Aldbrough Beck	54°30'15"N 01°41'51"W	150.7	5.4 ± 0.8	3.9 ± 1.2	Uninvaded
18	Clow Beck	54°29'21"N 01°37'21"W	166.3	6.2 ± 0.6	6.2 ± 0.6	Uninvaded

[1] Based on signal crayfish invasion status. Invaded (before 2011), sites where signal crayfish invaded before 2011, invaded (since 2011), invaded by signal crayfish between 2011 and 2018, and uninvaded, streams with no signal crayfish recorded or known.

The second study S_2 was conducted in Thorsgill and Alwent Becks in 2018. Both are within the list of streams considered in the first study (S_1). Fish and macroinvertebrate surveys were carried out at signal crayfish invaded and uninvaded sections within the same stream close in time and in the same conditions. In S_3, long-term water quality and macroinvertebrate data of six streams obtained from the Environment Agency, England (also overlaps with streams surveyed in S_1) were analysed. Therefore, combinations of these three studies can effectively reveal the impacts of non-native signal crayfish on native fish and macroinvertebrate communities in upland streams on different temporal and spatial scales.

A BACI approach was employed in S_1 and S_3 (Boys *et al.*, 2012; Galib, Lucas, *et al.*, 2018; Galib, Mohsin, *et al.*, 2018) and a CI approach in S_2 where sampling years represent time 'Before-After (BA)' and status of signal crayfish (present or absent) in streams represents 'control' (i.e. uninvaded streams) and 'impact' (i.e. invaded streams) sites.

4.2.2 Methods

4.2.2.1 Fish and signal crayfish sampling

In the first study S_1, fish, signal crayfish and benthic macroinvertebrates were sampled in summer 2011 and 2018 at the same site for each stream during base-level water flows. A wetted area of between 97.8 and 212.2 m^2 comprising riffle/cascade, glide and pool habitat was surveyed at each site (Table 4.1). Fish densities were estimated from depletion sampling (three runs, minimum period between runs, 15 minutes) using electrofishing by wading (pulsed DC current, Electracatch WFC4, Honda EU inverter 10i generator). Stop nets were placed at the boundaries of the sampling reach. After each electrofishing run fish species were identified, counted and their lengths were measured before releasing them outside of the fished area. Densities were calculated by the method of Carle and Strub (1978). In two signal crayfish invaded streams, Thorsgill Beck and Alwent Beck, fish sampling was also carried out at sites upstream of the invasion front in order to compare fish populations between invaded and non-invaded parts

of the same stream. In S_2, fish densities were estimated at both crayfish invaded and uninvaded sections of Thorsgill Beck and Alwent Beck employing the electrofishing method described above.

In both S_1 and S_2, signal crayfish, if present, were caught during electrofishing for fish and by subsequent refuge-searching methods using hand-nets in the same survey areas, because electrofishing only is not a sufficiently quantitative method for crayfish survey in rocky streams, even if it can be useful for determining presence vs. absence (Cowx and Lamarque, 1990; Gladman *et al.*, 2010). Manual searching of potential refuges and crayfish capture by hand-net (Bubb *et al.*, 2005), was carried out for one hour (by one experienced person) or 30 minutes (by two experienced persons), after electrofishing, covering a full range of sediment sizes available and used by crayfish. Crayfish catches from electrofishing and refuge searching were combined in order to calculate the minimum density of crayfish at each sampling site per standard unit of effort. Although standardised in format, the sampling did not allow population estimation. All crayfish capture methods over large areas are size selective and undersample YoY (<10 mm CL). But a combination of manual searching and electrofishing can be advantageous in measuring signal crayfish population size structure (Wutz and Geist, 2013), whereas, other commonly employed methods suffered major bias. For example, crayfish trapping commonly leads to a bias, with a higher probability of catching larger male individuals and under representation of female and young ones (Wutz and Geist, 2013). Crayfish were identified, measured (carapace length, CL) using Vernier slide calipers, and sexed (for crayfish with CL>10 mm).

4.2.2.2 Macroinvertebrate sampling

Macroinvertebrate samples were collected at fished sites in 2011 and 2018 by 3-minute kick sampling covering all available habitats, and an additional 1-minute, detailed hand search (Murray-Bligh, 1999). Immediately after collection, samples were preserved in 70% ethanol. In the laboratory, invertebrates were identified to family level, except Oligochaeta, Tipuloidea (including Tipulidae, Pediciidae and Limoniidae), Rhyacophilidae (including those species now often separated as Glossosomatidae) and Hydracarina,

following standard identification literature used in Chapter Two (p. 55). This was required in 2018, to match the taxonomic resolution used in 2011. In two signal crayfish invaded streams (Alwent Beck and Thorsgill Beck; in the second study, S_2), quantitative macroinvertebrate samples were collected from invaded and uninvaded sections (*N* = 6 in each section) of the streams using a 0.1 m^2 Surber sampler.

Although duplicated sampling in 2011 and 2018 (S_1), at the same 18 sites, provided BACI data with spatial replication across three invasion conditions (uninvaded, invaded (since 2011), invaded (before 2011)), it gave limited temporal context. Therefore, in S_3, long-term (since 1990) macroinvertebrate sampling data were analysed (spring and autumn only, because of larger sample size in these seasons compared to others; *N* = 162 total) for six of the Tees tributaries in the 2011 and 2018 sampling dataset (Albdrough Beck, Clow Beck, River Greta [*N* = 3 uninvaded sites] and Deepdale Beck, River Balder, River Lune [*N* = 3 invaded sites] – see Figure 4.1). These data were obtained from the Environment Agency (EA), England, and employed the same standardised kick sampling methods as described above. The signal crayfish establishment periods in invaded streams at my sample sites, and at the EA sampling localities were identified as 1995–2000 for the Balder and Deepdale Beck, and 2012–2014 for the Lune (MC Lucas; pers. comm.). Approximate mid-points of these years were employed as the invasion year during analysis, i.e. mid-1997 (for Deepdale and Balder) and 2013 (for Lune). Similar taxonomic resolution in analysis of EA data was used as described above except that in EA data oligochaetes were resolved to family level and Glossosomatidae and Rhyacophilidae were separated.

4.2.2.3 Water quality parameters and habitat characteristics

In 2011 and 2018 habitat characteristics were recorded at each site. Flow velocity was measured with an electromagnetic flow meter (Valeport 801, UK) through a series of transects at 60% depth of water (25%, 50% and 75% width positions of the stream, across transects at the downstream end, upstream end and positions 25%, 50% and 75% along the sample section; *N* = 15 at each sampling site). Water depth was recorded at the same stream channel locations as for flow velocity. The percentage area

covered by each of four water-flow types (Semple, 1991), cascade, riffle, glide and pool, at each survey section was recorded.

A simplified version of the Wentworth Scale (Wentworth, 1922), described in Chapter Two (p. 53), was used to measure the percentage cover of substrates, estimated by eye. The percentage of embedded substrate (pebble, cobble, boulder) which had to be pulled free from sediments on the river bed) was estimated at each sampling site. A canopy cover scale (measured as a discrete semi-quantitative scale, see Table S4.1 for details; modified from Ream, 2010) was used to estimate shading on each site of the stream. Due to the hydrological pattern and substrate conditions, Tees tributaries have few macrophytes; instream vegetation is dominated by diatoms and river moss *Fontinalis* attached to boulders/bedrock; although instream plant coverage was recorded, it varied relatively little and was not included in analyses.

Available historical (since 1990) water quality data (water temperature, turbidity, biochemical oxygen demand [BOD], dissolved oxygen [DO], pH, ammonia, total nitrogen, total hardness and zinc) for several control and invaded streams in the long-term invertebrate study element S_3 were obtained from the EA. These allowed comparison of changes in water quality over a long period across streams in the study area.

4.2.3 Statistical tests

Linear Mixed-Effects Modelling (LMM), described in Chapter Two (p. 62) was employed to analyse repeated measures fish density data. However, two invaded stream categories in S_1 (invaded since and before 2011) were analysed separately. During LMM, sampling years (i.e. period, before vs. after) was tested as a fixed effect, and sampling streams were considered a random effect. LMMs were also employed to determine changes in stream habitat characteristics (i.e. bottom substrate, depths, flow typology and shading; using percent data for bottom substrate and flow typology; Crawley, 2013). Temporal changes (2011 vs. 2018) in various groups of interest were determined by calculating effect size, Hedges' *g* (Hedges, 1981) using the statistical package 'effsize' in R, described in Chapter Two

(p. 63**).** Fish length data collected in 2011 and 2018 were compared using the non-parametric Mann-Whitney *U* test.

A Non-Metric Multidimensional Scaling (NMDS; Kruskal and Wish, 1978) ordination plot was generated to visualize spatial and temporal variation of fish community composition, based on abundance data, using the "metaMDS" function of the "vegan" package (Oksanen *et al.*, 2018). NMDS plots were also generated, based on presence or absence of invertebrate families (Royle and Nichols, 2003), for macroinvertebrate communities, based on Biological Monitoring Working Party (BMWP) scoring families (see Armitage *et al.*, 1983 for list), in 2011 and 2018. To determine the dissimilarities among fish and macroinvertebrate communities a PERMANOVA (described in Chapter Two, p. 62**)**. NMDS and PERMANOVA were applied only to the uninvaded and invaded (<2011) stream data due to the small sample size for newly-invaded streams (*N* = 3 per year).

SIMPER tests, described in Chapter Two (p. 62), were used to determine the average percent dissimilarity over time (2011 [before] vs. 2018 [after]) and to identify the contribution of individual fish species, belonging to each stream category, responsible for average dissimilarity between 'before' and 'after' communities. SIMPER was also employed to analyse macroinvertebrate data, based on presence or absence of families, collected in 2011 and 2018.

In S_1, as both fish and environmental data were available, the multivariate BIOENV procedure, based on Euclidean distances (Clarke and Ainsworth, 1993), was employed to find out the best subset of environmental variables with maximum (rank) correlation (Pearson's) with community dissimilarities (e.g. Boys *et al.*, 2012; Galib, Lucas, *et al.*, 2018; Galib, Mohsin, *et al.*, 2018). Along with all the environmental variables (depth and flow of water and habitat characteristics) density of signal crayfish was also considered in the BIOENV model to determine the role of signal crayfish for changes in fish community over time.

In S_3, for three uninvaded streams (Aldbrough, Clow and Greta), macroinvertebrate samples collected until mid-1997 were considered

'before' and samples collected after 1997 were considered 'after' situations. Four families were pooled because of variations in some aspects of taxonomic resolution through time (Limoniidae and Pediciidae were grouped under Tipuloidea; Lumbricilidae and Lumbricidae were pooled as Oligochaeta; after Durance and Ormerod, 2009). Nematoda and Hydracarina were recorded at that taxonomic resolution. As actual abundance data of macroinvertebrates were recorded on a ranked scale of logarithmic abundance, they were transformed on an ordinal scale (1 = 1–9 individuals, 2 = 10–99, 3 = 100–999, and 4 = 1000–9999) before analysis (after Durance and Ormerod, 2009) and used in PERMANOVA, NMDS and SIMPER.

Due to variations in macroinvertebrate samplings across studies, all relevant community analyses were based on presence or absence data (Royle and Nichols, 2003) based on biological monitoring working party [BMWP] scoring families (see Armitage *et al.*, 1983 for list) in S_1, whereas abundance and categorical data were used for S_2 and S_3 respectively.

Before-after changes in water quality parameters were determined separately for each stream category (in S_1) and stream (in S_3) to better understand the changes in individual category or stream using LLMs with sampling stream (S_1) and months (S_3, nested within year) as random effects. All statistical tests were carried out in R (version 3.4.3; R Core Team, 2017), with an α level of significance of 0.05. Data were explored, tested for normality and transformed following the methods described in Chapter Two (section 2.2.3, p. 61).

4.3 Results

4.3.1 Fish and signal crayfish

In the first study S_1, that represents a change over a seven-year period, no significant changes occurred in fish community composition, abundance or richness between uninvaded and newly-invaded streams (Table 4.2). Comparison between uninvaded and pre-invaded streams revealed

significant time and location effects on the fish community and abundance respectively (Table 4.2).

Table 4.2 Before-After-Control-Impact (BACI) and Control-Impact (CI) comparison of fish communities over time and space, obtained through Permutational Multivariate Analysis of Variance (PERMANOVA, for community) and Linear Mixed-Effects Modelling (LMM, for abundance and richness).

Comparisons	Community		Abundance		Richness	
	F	***P***	***F***	***P***	***F***	***P***
Study I (S_1) (Uninvaded vs. Newly-invaded streams)						
Time (BA)	0.5	0.671	0.5	0.517	1.0	0.342
Location (CI)	0.5	0.652	0.5	0.474	1.2	0.300
Interaction (BA×CI)	0.6	0.681	0.5	0.503	0.1	0.865
Study I (S_1) (Uninvaded vs. pre-invaded streams)						
Time (BA)	0.9	**0.043**	0.8	0.384	1.4	0.265
Location (CI)	2.3	0.176	10.8	**0.006**	0.2	0.712
Interaction (BA×CI)	0.1	0.774	1.0	0.343	0.01	0.965
Study II (S_2) (Uninvaded vs. invaded stretches)						
Location (CI)	0.6	0.500	254	**0.039**	1.0	0.500

Pairwise comparisons showed that mean fish abundance declined by 29% in pre-invaded streams between 2011 and 2018 (P = 0.027; Table 4.3) but abundance did not change in uninvaded control streams. Mean crayfish density increased by 93.1% in pre-invaded streams between 2011 and 2018 (P = 0.019, Figure 4.2), over the same period that abundance of YoY salmonids (P = 0.038) and small benthic fish (bullhead and stone loach, P = 0.022; Table 4.3) decreased by ~30% and ~85% respectively (Figure 4.2). However, abundance of non-YoY salmonids increased by over 100% in both pre- and newly- invaded streams. The overall mean minimum (± SD) density of signal crayfish in pre-invaded streams was 46.4 ± 31.5 crayfish $100m^{-2}$ in 2011 and 89.7 ± 50.4 crayfish $100m^{-2}$ in 2018. The mean minimum density in newly-invaded streams was 31.4 ± 22.5 crayfish $100m^{-2}$ (Table 4.4).

TABLE 4.3 Statistical comparisons of abundance of fishes and signal crayfish over time (2011 vs. 2018) in relation to stream invasion status by signal crayfish, obtained through Linear Mixed-Effects Modelling (LMM).

Groups	***Occurrence (n)***	***F***	***P***	***Mean changes in abundance (%)***
Pre-invaded streams (*n* = 8)				
Overall fishes	8	7.71	**0.027**	↓ 29
Signal crayfish	8	9.30	**0.019**	↑ 93.1
YoY salmonids	4	12.59	**0.038**	↓ 31.7
Benthic fishes	7	6.95	**0.022**	↓ 83.2
Uninvaded streams (*n* = 7)				
Overall fishes	7	0.09	0.776	↑ 21.1
YoY salmonids	7	0.28	0.616	↑ 4.4
Benthic fishes	5	0.01	0.941	↑ 29.7
Newly-invaded streams (*n* = 3)				
Overall fishes	3	5.65	0.141	↓ 54.3
YoY salmonids	2	1.64	0.399	↓ 61.5
Benthic fishes	3	5.87	0.136	↓ 61.3

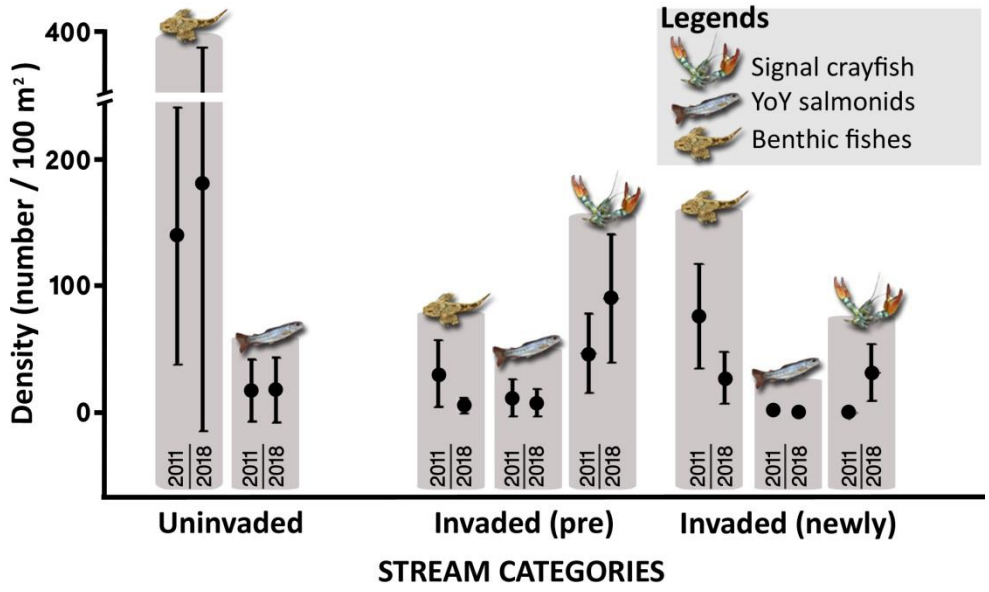


FIGURE 4.2 Changes in density (individuals 100 m^{-2}) of bullhead, YoY (young-of-year) salmonids and signal crayfish, (mean ± SD) between 2011 and 2018 across sites at different invasion stages. Fish densities measured by depletion sampling, crayfish are minimum densities per standardised effort (see text for more detail).

TABLE 4.4 Density (mean ± SD, 100m^{-2}) of signal crayfish and other fish species and groups in 2011 and 2018.

Species / group	Pre-invaded streams (*N* = 8)			Newly-invaded streams (*N* = 3)			Uninvaded streams (*N* = 7)		
	N	2011	2018	*N*	2011	2018	*N*	2011	2018
Signal crayfish	8	46.4± 31.5	89.7± 50.4	3	0	31.4± 22.5	-	-	-
Bullhead	5	30.5± 26.4	4.9± 6.4	3	75.9± 41.2	30.3± 18.4	5	139.4± 101.6	180.7± 195.6
Brown trout	7	9.1± 17.7	12.7± 14.8	3	1.6± 0.8	5.3± 3.4	7	21.7± 24.4	23.3± 28.6
Minnow	6	3.2± 4.9	3.2± 4.8	2	9.6± 11.6	12.7± 13.4	2	9.6± 13.6	14.8± 13.7
Stone loach	3	4.7± 3.6	1.1± 1.2	2	3.8± 4.3	0	1	4.0	5.1
Grayling	1	0.6	0	-			1	0.6	0
Atlantic salmon	-	-	-	1	2	1.6	2	4.6± 6.5	9.8± 13.9
3-spined stickleback	2	10.4± 10	28.0± 39.5	1	9.6	5.5	2	26.3± 33.4	6.4± 9.0
Lamprey	-	-	-	1	37.0	1.20	2	2.1± 2.1	0.9± 0.4
Eel	-	-	-				1	0	1.9
Roach	-	-	-				1	0.6	0
Total	8	33.8± 28.1	24.0± 19.9	3	102.6± 66.7	46.9± 30.2	7	134.2± 107.1	162.4± 176.9
Benthic fishes	8	20.8± 24.4	3.5± 5.3	3	78.4± 43	30.3± 18.4	5	140.2± 100.9	181.7± 194.9
Non-benthic	8	13± 14.8	20.5± 20.9	3	24.2± 35.5	16.5± 12.2	7	34± 29.8	32.6± 23.5
YoY salmonids	4	11± 14.6	7.5± 10.8	2	1.3± 0.0	0.5± 0.7	7	16.7± 24.4	17.5± 25.2
Other salmonids	7	2.8± 5.0	8.4± 6.9	3	0.7± 0	4.0± 4.0	7	5.0± 4.6	5.8± 7.2

N, number of streams where species was recorded, Total, total density (all species); Benthic fishes, bullhead and Stone loach.

Abundance of bullhead has declined in both signal crayfish invaded stream categories, by 83.8% in pre-invaded and by 60% in newly-invaded streams (Table 4.5). Nonetheless, their abundance has increased, by 29.7%, in uninvaded streams (Table 4.5). Bullhead were responsible for the majority of the change in fish species assemblages between 2011 and 2018 (52%, 31% and 32.3% in un–, pre–, and newly– invaded streams respectively), followed by brown trout and minnow (Table 4.6). Only in pre-invaded streams the change in bullhead relative abundance was significant in contributing to community change (P = 0.019), accompanied by an increase in relative abundance of trout (P = 0.024). Divergence in fish community composition following crayfish invasion is evident from the NMDS ordination plot (Figure 4.3).

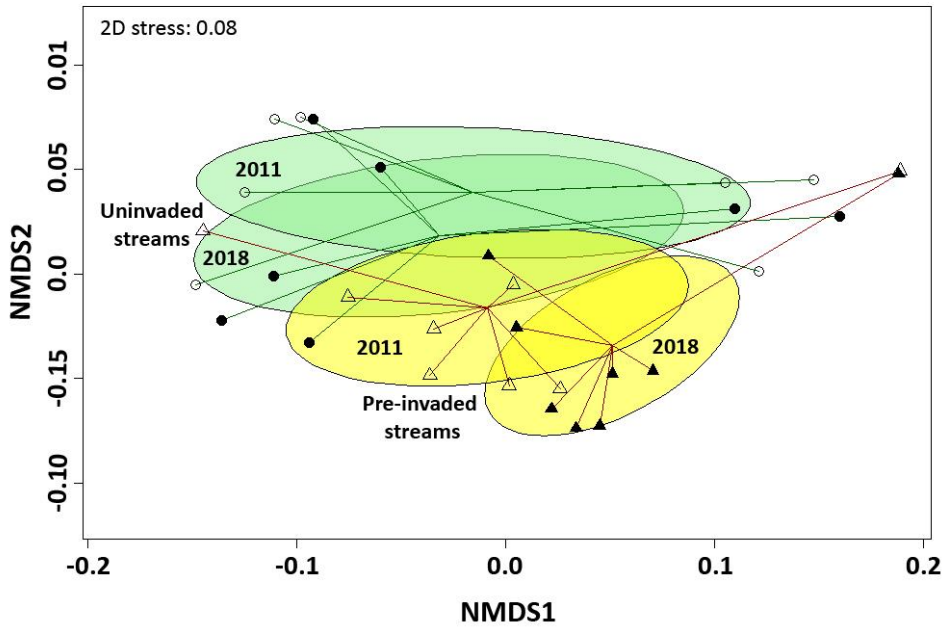


FIGURE 4.3 Non-Metric Multidimensional Scaling (NMDS) ordination plots showing spatial and temporal variation of fish communities between pre-invaded and uninvaded streams. Ellipses represent 95% confidence interval.

TABLE 4.5 Before-After comparison of fish species abundance over time (2011 vs. 2018) in studied streams belonging to different categories in relation to signal crayfish invasion, obtained through Linear Mixed-Effects Modelling (LMM).

Fish / group	*Family*	*Before vs. After*			*Change in abundance (%)*
		n	*F*	*P*	
Pre-invaded streams (*n* = 8)					
Bullhead	Cottidae	5	8.6	**0.019**	↓ 83.8
Brown trout	Salmonidae	7	9.0	**0.024**	↑ 39.2
Minnow	Cyprinidae	6	0.01	0.938	↑ 2.1
3 spined stickleback	Gasterosteidae	2	0.1	0.820	↑ 170.1
Stone loach	Nemacheilidae	3	2.3	0.204	↓ 76.4
Grayling	Salmonidae	1	-	-	↓ 100
Uninvaded streams (*n* = 7)					
Bullhead	Cottidae	5	0.01	0.944	↑ 29.7
Brown trout	Salmonidae	7	0.03	0.860	↑ 7.2
3-spined stickleback	Gasterosteidae	2	0.6	0.511	↑ 75.9
Atlantic Salmon	Salmonidae	2	0.1	0.819	↑ 113.04
Minnow	Cyprinidae	2	2.7	0.346	↑ 53.7
Stone loach	Nemacheilidae	1	-	-	↑ 27.5
Lamprey	Petromyzontidae	2	1.0	0.498	↑ 57.1
Eel	Anguillidae	1	-	-	↑ 100
Grayling	Salmonidae	1	-	-	↓ 100
Roach	Cyprinidae	1	-	-	↓ 100
Newly invaded streams (*n* = 3)					
Bullhead	Cottidae	3	6.2	0.131	↓ 60.0
Minnow	Cyprinidae	2	18.5	0.145	↑ 31.8
Lamprey	Petromyzontidae	1	-	-	↓ 96.8
Stone loach	Nemacheilidae	2	3.8	0.287	↓ 100
Brown trout	Salmonidae	3	6.1	0.133	↑ 241.2
3-spined stickleback	Gasterosteidae	1	-	-	↓ 42.7
Atlantic salmon	Salmonidae	1	-	-	↓ 20

TABLE 4.6 Before-After comparison of fish species community condition, accounting for differences over time (2011 vs. 2018) in studied streams belonging to different categories in relation to signal crayfish invasion, obtained through Similarity Percentage Analysis (SIMPER).

Fish / group	*Ratio*	*Average*	*Average abundance*		*P-values*	*Cumulative contribution*
			2011	*2018*		
Pre-invaded streams (*n* = 8)						
Bullhead	1.24	0.20	3.24	1.00	0.163	31
Brown trout	1.12	0.18	1.93	2.79	0.400	58
Minnow	1.05	0.09	0.93	0.98	0.967	72
3-spined stickleback	0.58	0.09	0.75	0.93	0.852	86
Stone loach	0.77	0.08	0.74	0.31	0.271	98
Grayling	0.36	0.01	0.10	0.00	0.132	100
Uninvaded streams (*n* = 7)						
Bullhead	1.34	0.28	7.75	7.92	0.749	52
Brown trout	1.42	0.11	3.91	4.06	0.882	72
3-spined stickleback	0.66	0.04	1.24	0.51	0.814	80
Atlantic Salmon	0.55	0.04	0.43	0.63	0.560	86
Minnow	0.70	0.03	0.63	1.03	0.884	92
Stone loach	0.54	0.02	0.29	0.32	0.620	96
Lamprey	0.81	0.01	0.38	0.27	0.811	98
Eel	0.40	0.00	0.00	0.20	0.675	99
Grayling	0.40	0.00	0.11	0.00	0.663	100
Roach	0.40	0.00	0.11	0.00	0.663	100
Newly invaded streams (*n* = 3)						
Bullhead	1.89	0.1438	8.43	4.96	0.200	32
Minnow	1.22	0.0778	1.80	2.16	1.000	50
Lamprey	0.79	0.0644	2.03	0.37	0.600	64
Stone loach	1.11	0.048	0.00	0.75	0.100	75
Brown trout	1.28	0.0439	1.22	2.21	0.300	85
3-spined stickleback	0.90	0.0421	1.03	0.78	0.900	95
Atlantic salmon	0.83	0.0246	0.47	0.42	0.900	100

There was no significant difference in fish communities between pre-invaded and uninvaded streams in 2011 (PERMANOVA, $P = 0.11$) but they varied significantly in 2018 ($P = 0.002$; Table 4.7). Fish community varied significantly in pre-invaded streams between 2011 and 2018 ($P = 0.048$) but did not in uninvaded streams (Table 4.7).

Table 4.7 Pairwise comparisons of fish community data over time and space in S_1 (2011 vs. 2018 data), obtained through Permutational Multivariate Analysis of Variance (PERMANOVA).

Comparisons	Time	Mean square	*df*	*F*	*P*
Uninvaded vs. pre-invaded	Before	0.317	1, 13	1.34	0.231
	After	0.901	1, 13	3.9	**0.002**
Uninvaded vs. newly-invaded	Before	0.260	1, 8	1.4	0.236
	After	0.272	1, 8	1.4	0.265
Uninvaded streams	Before vs. After	0.017	1, 12	0.08	0.969
Pre-invaded streams	Before vs. After	0.567	1, 14	2.21	**0.048**
Newly-invaded streams	Before vs. After	0.111	1, 4	1.0	0.600

Pairwise effect size analyses also confirmed negligible to small temporal effect size in uninvaded streams, but small to large effect size in newly- and pre- invaded streams (Table 4.8). No bullhead were found in two pre-invaded streams (Lance Beck and Westholme Beck) in 2018 where they were abundant in 2011. Fish abundance differed between invaded and uninvaded sections of two streams in 2018 (Thorsgill and Alwent becks; $F = 145.2$, $P = 0.034$). Higher fish abundance (by >110%) occurred in uninvaded upstream sites compared to sites invaded by signal crayfish.

In pre-invaded streams, the proportion of crayfish categorised as large (≥35 mm CL) increased from 18.4% (mean [± SD] minimum density and range: 8.2 ± 5.9 $100m^{-2}$, 1.9–18.6 $100m^{-2}$) in 2011 to 24.1% in 2018 (mean [± SD] minimum density and range: 19.8 ± 25.1 $100m^{-2}$, 2.25–72.9 $100m^{-2}$; Figure 4.4). By contrast, the proportion of large crayfish was 26.4% in newly invaded streams (mean [± SD] minimum density and range:

8.3 ± 6.1 100m^{-2}, 4–15.3 100m^{-2}; Figure 4.4). Sex ratio (male : female) of signal crayfish in pre-invaded streams was 1 : 1.12 and 1 : 1.27 in 2011 and 2018 respectively and was 1 : 1.15 in newly invaded streams (2018), based on a total of 1053 sexed crayfish. Out of eight pre-invaded streams in 2018, a reduced minimum density of signal crayfish (34.5 crayfish 100m^{-2}) was only recorded in Scur Beck, compared to the minimum density recorded in 2011 (62.8 crayfish 100m^{-2}).

Table 4.8 Comparisons of abundance of fishes in different streams categories, based on electrofishing survey data obtained in S_1 (2011 vs. 2018).

Groups	Stream categories	Effect size (Hedges' *g*)		
		2011 vs. 2018	Effect type	95% confidence interval
Overall fish abundance	Uninvaded	–0.18	Negligible	–1.28 to 0.91
	Newly-invaded	0.90	Large	–1.00 to 2.81
	Pre-invaded	0.45	Small	–0.57 to 1.48
YoY salmonids	Uninvaded	–0.03	Negligible	–1.12 to 1.06
	Newly-invaded	0.91	Large	–1.67 to 3.50
	Pre-invaded	0.24	Small	–1.27 to 1.75
Benthic fishes	Uninvaded	–0.24	Small	–1.56 to 1.08
	Newly-invaded	1.22	Large	–0.76 to 3.19
	Pre-invaded	1.05	Large	–0.11 to 2.22

In uninvaded streams there was no difference in size (age) structure of bullhead between 2011 and 2018, with good recruitment of younger age groups into the population in both years (Figure 4.5). By contrast, in pre-invaded streams there was a highly significant difference (Mann-Whitney *U* test: $U = 4889.5$, $P < 0.001$) between 2011 and 2018, with negligible numbers of young and evidence of recruitment failure over several years up to and including 2018, during which time crayfish increased in

abundance. The same analysis for brown trout (Figure 4.6) showed no significant difference in size structure of trout between 2011 and 2018.

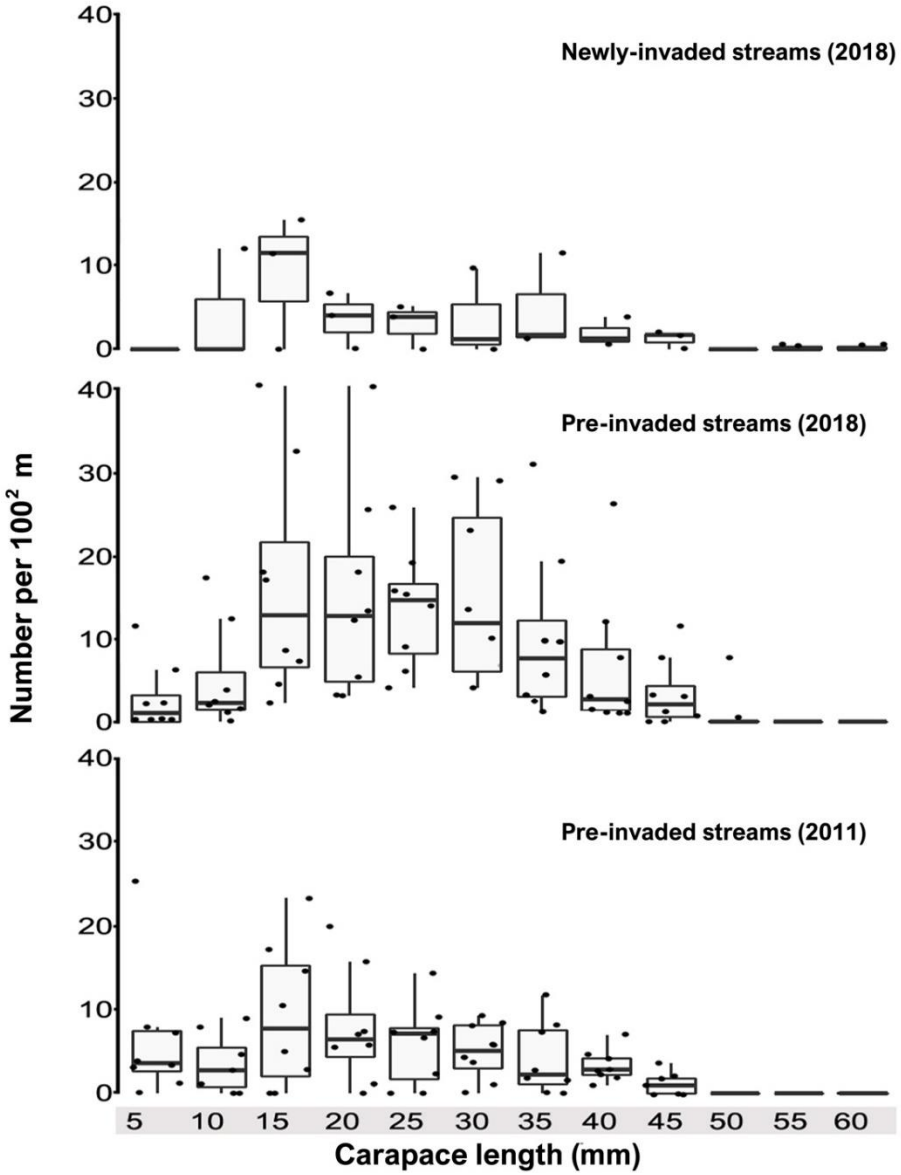


FIGURE 4.4 Boxplots showing minimum density of non-native signal crayfish, belonging to different size groups, in invaded Tees tributaries. Midline within the box is the median; upper and lower limits of the box represent the third and first quartile (75th and 25th percentile) respectively. Points are individual site data.

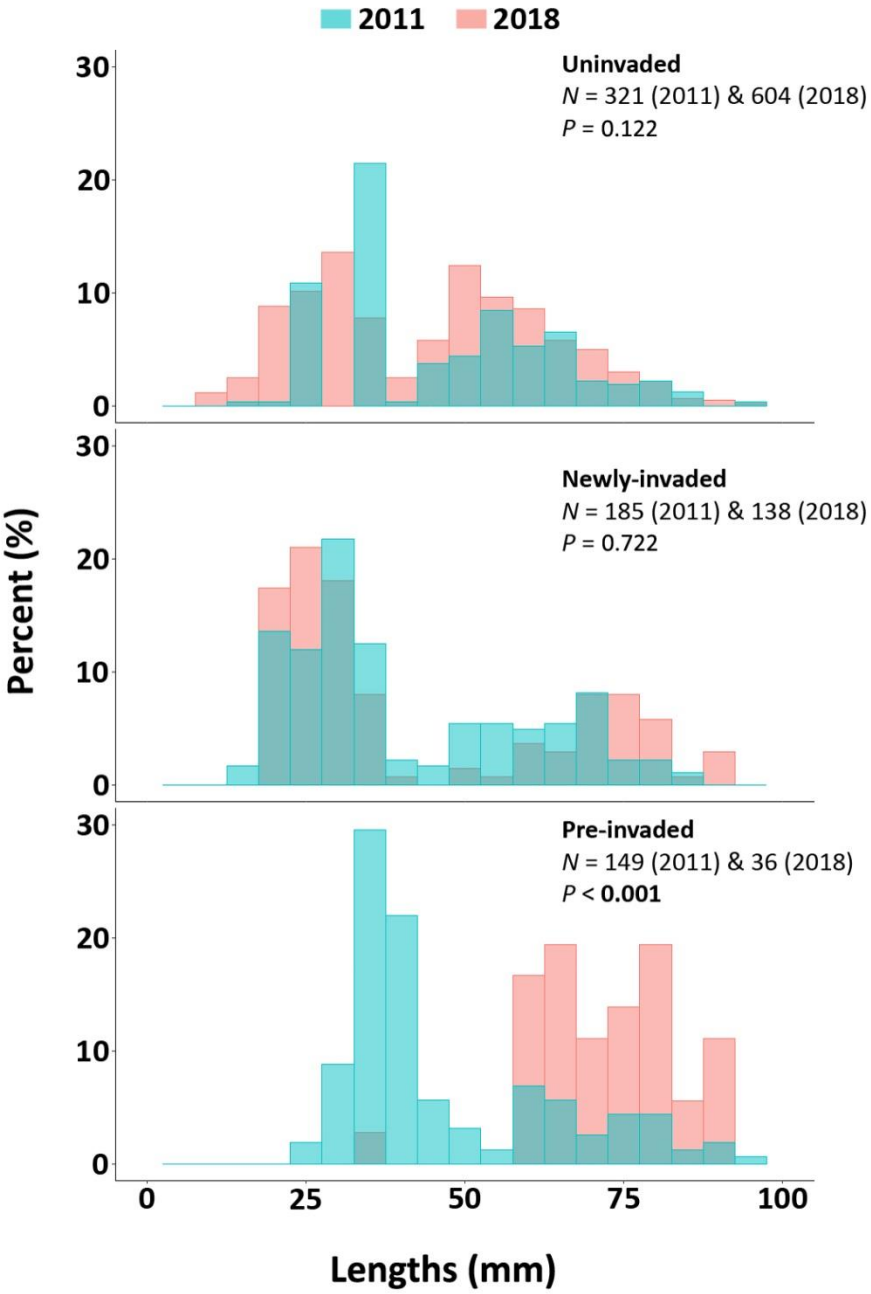


FIGURE 4.5 Total length of bullhead *Cottus gobio* recorded in two sampling years (2011 and 2018) in different stream categories.

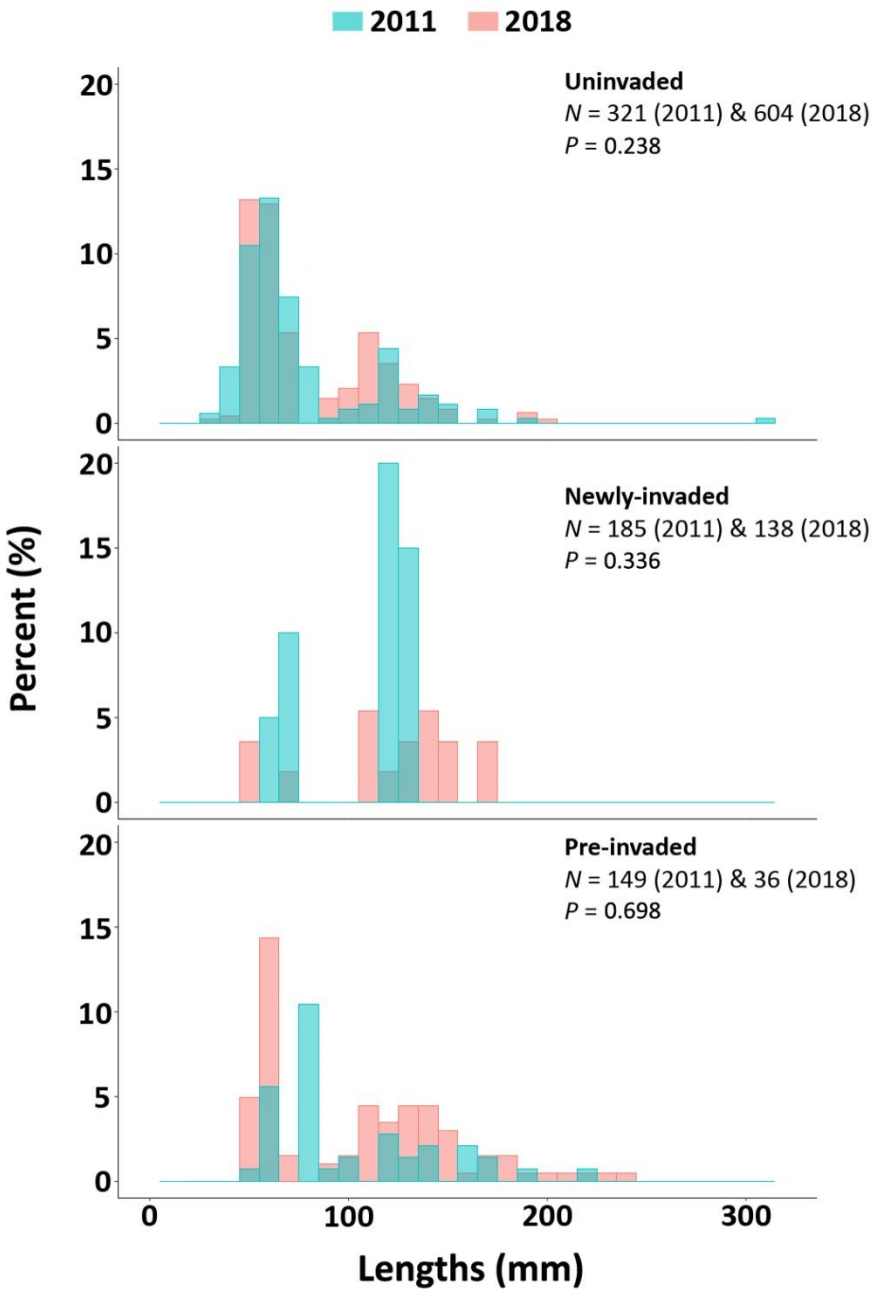


FIGURE 4.6 Total length of salmonids recorded in two sampling years (2011 and 2018) in different stream categories.

4.3.2 Macroinvertebrates

Negative impacts of signal crayfish on macroinvertebrates were recorded between uninvaded and invaded streams over medium- to long- terms (S_1

and S_3) and between locations sampled contemporaneously within the same stream (S_2).

4.3.2.1 Community differences between 2011 and 2018

Significant time, location and their interaction effects on macroinvertebrate communities were recorded between uninvaded and pre-invaded streams in the first study S_1 (all $P \leq 0.01$; Table 4.9). Comparing uninvaded and newly-invaded streams, only a location effect was significant ($P = 0.015$; Table 4.9).

Table 4.9 Before-After-Control-Impact (BACI) and Control-Impact (CI) comparisons of macroinvertebrate data over time and space, obtained through Permutational Multivariate Analysis of Variance (PERMANOVA, for community) and Linear Mixed-Effects Modelling (LMM, for abundance and richness).

Comparisons	Community		Abundance		Richness	
	F	***P***	***F***	***P***	***F***	***P***
Study I (S_1) (Uninvaded vs. newly-invaded)						
Time (BA)	1.6	0.140	–	–	–	–
Location (CI)	2.6	**0.015**	–	–	–	–
Interaction (BA×CI)	0.7	0.730	–	–	–	–
Study I (S_1) (Uninvaded vs. pre-invaded)						
Time (BA)	5.7	**0.005**	–	–	–	–
Location (CI)	3.5	**0.010**	–	–	–	–
Interaction (BA×CI)	0.9	**<0.001**	–	–	–	–
Study II (S_2)						
Location (CI)	5.9	**0.010**	33.6	**<0.001**	17.0	**<0.001**
Study III (S_3)						
Time (BA)	6.6	**<0.001**	–	–	0.6	0.457
Location (CI)	18.3	**<0.001**	–	–	4.6	**0.05**
Interaction (BA×CI)	4.2	**<0.001**	–	–	8.4	**0.004**

The invertebrate communities recorded in both 2011 and 2018 differed significantly between pre-invaded and uninvaded streams (PERMANOVA, 2011: $F = 2.8$, $P = 0.013$; 2018: $F = 3.9$, $P < 0.001$; Table 4.10). The community differed significantly between years in pre-invaded

streams ($P < 0.001$) whereas it did not in uninvaded streams (Table 4.10), reflecting an ongoing trajectory of separation in community characteristics between invaded and uninvaded streams (Figure 4.7).

Table 4.10 Pairwise comparisons of macroinvertebrate data over time and space in S_1 (2011 vs. 2018 data) and S_3 (1990–2017 data), obtained through Permutational Multivariate Analysis of Variance (PERMANOVA, for community) and Linear Mixed-Effects Modelling (LMM, for richness and abundance).

Study and group	Comparison	Time	*F*	*P*
Study I (S_1); Macroinvertebrate community	Uninvaded vs. invaded (pre)	Before	2.8	**0.013**
		After	3.9	**<0.001**
	Uninvaded vs. invaded (newly)	Before	1.9	0.072
		After	2.3	**0.048**
	Uninvaded streams	Before vs. After	1.7	0.143
	Invaded (pre) streams	Before vs. After	2.8	**0.008**
	Invaded (newly) streams	Before vs. After	0.6	0.700
Study III (S_3) Macroinvertebrate community	Uninvaded vs. invaded	Before	2.0	0.070
		After	15.4	**<0.001**
	Uninvaded streams	Before vs. After	5.3	**0.001**
	Invaded streams	Before vs. After	5.7	<**0.001**
Study III (S_3) Macroinvertebrate taxonomic richness	Uninvaded vs. invaded	Before	0.1	0.726
		After	5.9	**0.041**
	Uninvaded streams	Before vs. After	0.2	0.638
	Invaded streams	Before vs. After	4.3	**0.047**

SIMPER analyses, based on 2011 and 2018 data, revealed that occurrence of three families differed significantly in the pre-invaded streams over time (all $P < 0.05$) including an increase in two families (Rhyacophilidae and Astacidae), and a decrease in Tipuloidea (Table S4.2). However, no such change was observed in newly-invaded streams (Table S4.3), although the sample size was small ($N = 3$ each year) in this

case. In uninvaded streams, occurrence of five taxa changed significantly (all $P < 0.05$) including an increase in three taxa (Polycentropidae, Erpobdeliidae and Oligochaeta; Table S4.4).

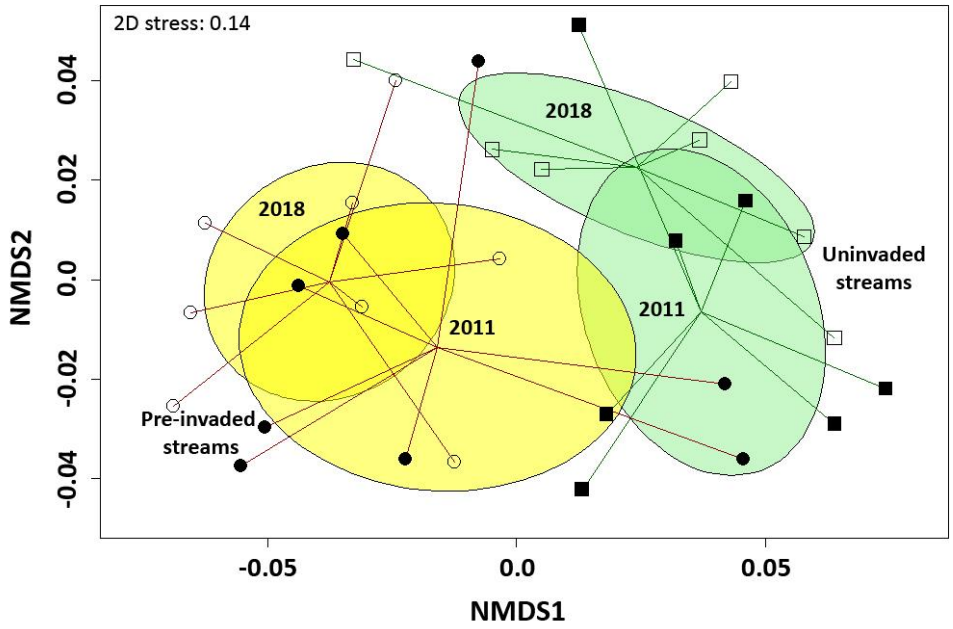


FIGURE 4.7 Non-Metric Multidimensional Scaling (NMDS) ordination plots showing spatial and temporal variation of macroinvertebrate communities between pre-invaded and uninvaded streams, based on presence-absence data. Ellipses represent 95% confidence interval.

4.3.2.2 Invaded vs. uninvaded sections within streams

Macroinvertebrate taxonomic richness and abundance differed significantly between crayfish–invaded and uninvaded sections of Thorsgill and Alwent becks (LMMs: richness and abundance, both $P < 0.001$; Table 4.9). Higher macroinvertebrate abundance (by >125%) was recorded in uninvaded upstream sites (density: mean ± SD, 90.1 ± 50.2 individuals $0.1m^{-2}$; range, 48 – 237) compared to invaded sites (density: mean ± SD, 40.0 ± 17.1 individuals $0.1m^{-2}$; range, 17 – 74). High macroinvertebrate taxonomic richness was recorded in uninvaded upstream locations of Thorsgill and Alwent becks (mean ± SD, 15.1 ± 2.4 families, range 12–20 families) compared to invaded downstream sites (11.1 ± 2.5 families, range 7 – 14 families).

The macroinvertebrate community also differed significantly between signal crayfish– invaded and uninvaded sections (PERMANOVA: $F = 5.55$, $P = 0.001$; Figure 4.8). SIMPER analysis revealed that the abundance of Elmidae, Hydrobiidae, Ephemerellidae, Odontoceridae, Sphaeriidae, Psychodidae, Ancylidae, Valvatidae and Caenidae were significantly lower at signal crayfish invaded sections compared to uninvaded sections (all $P < 0.05$; Table S4.5). More than 50% of the differences in communities between uninvaded and invaded sites were because of eight macroinvertebrate families (Elmidae, Hydrobiidae, Gammaridae, Chironomidae, Ephemerellidae, Heptageniidae, Nemouridae and Rhyacophilidae; Table S4.5).

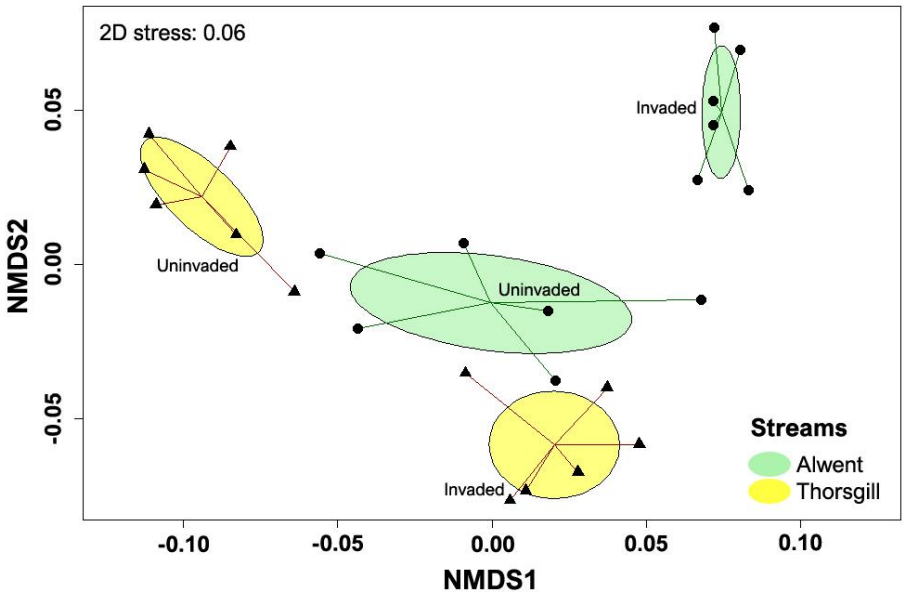


FIGURE 4.8 Non-Metric Multidimensional Scaling (NMDS) ordination plots showing spatial and temporal variation of macroinvertebrate communities between signal crayfish invaded and uninvaded parts within Alwent and Thorsgill becks, based on abundance data. Ellipses represent 95% confidence interval.

4.3.2.3 Long-term changes

For the long-term invertebrate data, highly significant time, location and interaction effects on macroinvertebrate communities were also recorded between uninvaded and invaded streams in the third study element S_3 (all $P < 0.001$; Table 4.9). Despite no difference in macroinvertebrate

taxonomic richness between invaded (*N* = 3) and uninvaded (*N* = 3) streams before signal crayfish colonisation, this differed significantly after invasion (*P* = 0.041, Table 4.10). Macroinvertebrates in invaded streams also differed significantly in taxonomic richness between the pre– and post–invasion period (*P* = 0.047) whereas invertebrates in uninvaded streams did not, over the equivalent periods (Table 4.10; Figure 4.9).

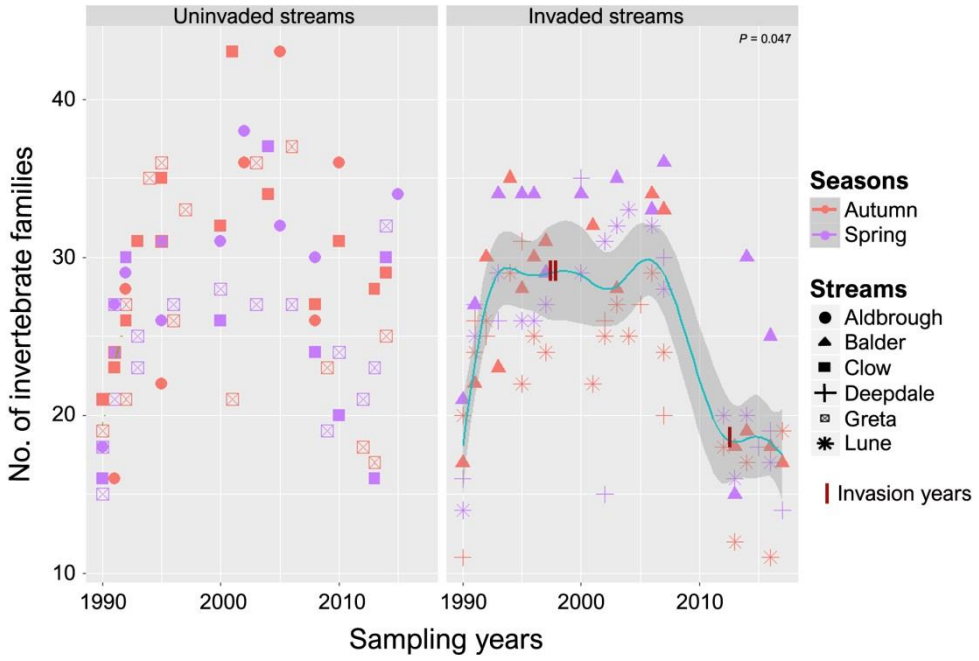


FIGURE 4.9 Historical trend of macroinvertebrate taxonomic richness in uninvaded streams (Aldbrough Beck, Clow Beck and River Greta) and invaded streams (Deepdale Beck, River Balder and River Lune) over time, smoothed fit with 95% confidence interval represented by grey-shaded areas (significant effect over time in invaded streams only, hence trend and 95% CL not shown for uninvaded streams).

The invertebrate community in invaded streams, compared to uninvaded streams, deviated more from its initial pre-invasion condition (NMDS, Figure 4.10). Similar to taxonomic richness, the macroinvertebrate communities of invaded and uninvaded streams did not differ before signal crayfish invasion (PERMANOVA: *P* = 0.070), but differed significantly after invasion (*P* < 0.001; Table 4.10). However, communities in both invaded and uninvaded streams changed significantly from before to after invasion (both *P* < 0.05; Table 4.10).

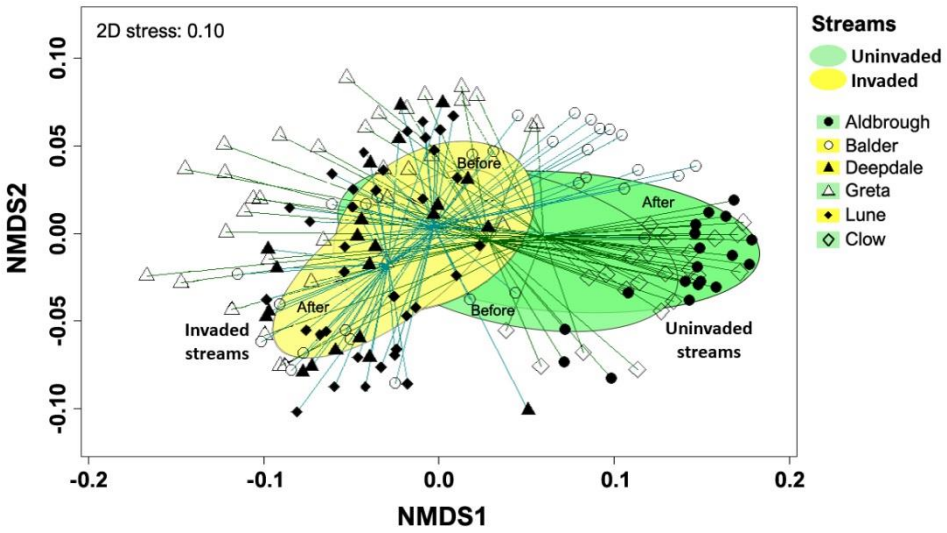


FIGURE 4.10 Non-Metric Multidimensional Scaling (NMDS) ordination plots showing spatial and temporal variation of macroinvertebrate communities before and after signal crayfish invasion, in three invaded and three uninvaded streams. Ellipses represent 95% confidence interval.

Conspicuous changes in community composition for both invaded and uninvaded streams occurred from before to after the invasion period (SIMPER, Table 4.11). In invaded streams a significant decrease (by 8–82%, all $P < 0.05$) in abundance of Lepidostomatidae, Caenidae, Ancylidae, Perlidae, Polycentropodidae, Limnephilidae, Leptophlebiidae, Sphaeriidae, Oligochaeta, Hydrobiidae, Gyrinidae, Rhyacophilidae and Hydropsychidae was recorded after invasion whereas they increased in uninvaded streams except for Perlidae, Polycentropodidae, Rhyacophilidae and Hydropsychidae that decreased by 41%, 46%, 16% and 2% respectively (Table 4.11 and Tables S4.6–S4.7).

A significant increase (all $P < 0.05$) in Hydrophilidae, Ephemerellidae, Glossosomatidae, Heptageniidae, Goeridae and Baetidae was recorded in both invaded streams and uninvaded streams except for Hydrophilidae, Heptageniidae (both decreased in uninvaded streams) and Ephemerellidae (absent in uninvaded streams) (Tables 4.11 & S4.7).

TABLE 4.11 Changes in top 10 macroinvertebrate families contributing to the dissimilarity in communities before and after signal crayfish invasion (1990–2017 data) along with several other families of concern in three invaded streams (Deepdale, Balder and Lune) and three uninvaded streams (Aldbrough, Clow and Greta) over the same period, obtained through Similarity Percentage Analysis (SIMPER) (also see Table S4.6–S4.7 for complete lists).

Macroinvertebrate families	Changes in abundance (%) from before to after invasion		Contribution to dissimilarity (%) from before to after invasion	
	Invaded streams	Uninvaded streams	Invaded streams	Uninvaded streams
Lepidostomatidae	↓ 55***	↑ 59*	3.3	2.6
Caenidae	↓ 47***	↑ 2	3.2	2.4
Ancylidae	↓ 58**	↑ 31	3.2	2.4
Perlidae	↓ 64**	↓ 41	3.1	2.0
Chloroperlidae	↑ 8	↓ 30	2.9	1.9
Sericostomatidae	↓ 15	↓ 3	2.8	2.0
Polycentropodidae	↓ 39**	↓ 46*	2.7	2.2
Limnephilidae	↓ 25*	↑ 23	2.7	2.4
Gammaridae	↑ 2	↑ 15	2.7	2.0
Leptophlebiidae	↓ 27***	↑ 53**	2.7	2.5
Sphaeriidae	↓ 38*	↑ 26	2.5	2.4
Hydrophilidae	↑ 91*	↓ 81*	2.3	1.3
Oligochaeta	↓ 19***	↑ 7	2.1	1.6
Hydrobiidae	↓ 82*	↑ 2	2.1	2.6
Gyrinidae	↓ 66*	↑ 11	2.2	2.0
Rhyacophilidae	↓18*	↓ 16	1.9	1.6
Ephemerellidae	↑ 285**	NA	1.8	NA
Glossosomatidae	↑ 306**	↑ 798***	1.7	3.7
Heptageniidae	↑ 9**	↓ 1	1.6	1.8
Goeridae	↑ 255**	↑ 387**	1.4	1.5
Baetidae	↑11**	↑ 12***	1.1	1.0
Hydropsychidae	↓ 8*	↓ 2	1.1	1.0
Perlodidae	↑ 11	↓ 32	2.6	2.5
Lymnaeidae	↓ 40	↑ 34	1.6	2.4
Glossiphoniidae	↓ 36	↑ 17	1.1	2.3
Erpobdellidae	↑ 29	↓ 1	0.3	2.3

***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ↑, increasing trend; ↓, decreasing trend; NA, absent

4.3.3 Habitat and physicochemistry of fish survey sites over time

No significant changes over time were recorded for habitat characteristics in the streams surveyed for fish communities (2011 vs. 2018; all $P > 0.05$; Table 4.12). Density of signal crayfish, and proportions of cascade and glide habitat collectively were most strongly correlated with the fish assemblage patterns observed in streams of the study area (BIOENV analysis Pearson correlation, $\rho = 0.42$). All the top 15 subset models with the highest correlations, out of 16383 combinations, contained signal crayfish. The density of signal crayfish alone was a strong predictor of fish community ($\rho = 0.34$).

TABLE 4.12 Status of habitat and parameters over time in S_1 (2011 vs. 2018) at BACI survey sites, obtained through Linear Mixed-Effects Modelling (LMM).

Parameters	Stream categories					
	Uninvaded		Newly-invaded		Pre-invaded	
	F	***P***	***F***	***P***	***F***	***P***
Water depth	1.14	0.327	2.39	0.262	0.69	0.433
Flow velocity	2.98	0.135	2.61	0.248	1.73	0.230
Bottom substrates						
Boulder	1.78	0.231	1	0.423	1	0.351
Cobble	4.50	0.078	0.68	0.498	2.15	0.186
Pebble	2.21	0.188	0.48	0.560	0.21	0.660
Gravel	0.94	0.370	0.08	0.802	0.06	0.810
Sand	5.26	0.062	0.40	0.594	5.25	0.056
Flow types						
Pool	1	0.360	0.08	0.802	1.75	0.228
Riffle	1.22	0.311	1	0.422	3.37	0.109
Glide	2.16	0.192	0.01	0.936	2.03	0.197
Cascade	1	0.356	1	0.423	2.27	0.176
Others						
Substrate embeddedness	2.4	0.172	0.14	0.742	1.57	0.23
Canopy cover	0.3	0.604	1	0.423	0	1

For long-term water quality data (Alwent, Lune, Balder, Deepdale, Greta, Clow), few water quality parameters in signal crayfish invaded streams varied significantly from before to after invasion (Table 4.13 and Figures S4.2–S4.10). Total nitrogen decreased significantly over time in the Balder (from 0.54 ± 0.17 to 0.41 ± 0.15 mgL^{-1}) and Lune (from 0.37 ± 0.15 to 0.27 ± 0.05 mgL^{-1}). Ammonia levels decreased significantly in Alwent Beck (from 0.05 ± 0.03 to 0.04 ± 0.05 mgL^{-1}) and the Balder (from 0.09 ± 0.17 to 0.04 ± 0.04 mgL^{-1}). BOD increased significantly over this period in Deepdale (from 1.76 ± 0.75 to 1.91 ± 0.69 mgL^{-1}) and the Lune (from 1.33 ± 0.55 to 1.92 ± 1.47 mgL^{-1}) but decreased in Alwent Beck (from 1.94 ± 0.93 to 1.59 ± 0.58 mgL^{-1}), but no changes in oxygen occurred in any stream. Total hardness decreased significantly in the Balder (from 49.63 ± 10.46 to 46.48 ± 10.18 mgL^{-1}). pH in Alwent Beck increased significantly from 8.09 ± 0.36 to 8.25 ± 0.23. Turbidity decreased significantly in the Balder (from 19.71 ± 38.62 to 4.03 ± 3.93 NTU), but increased slightly in the Lune (from 3.32 ± 2.90 to 4.50 ± 3.42 NTU; Table 4.13). In general, water quality (in terms of the needs of fish and benthic macroinvertebrates) tended to improve or remain stable across all streams over the period 1990–2018.

TABLE 4.13 Long-term comparison (1990 to 2018) of changes in water quality parameters over before and after signal crayfish invasion in invaded (*n* = 4; Alwent Beck, Deepdale Beck, River Balder and River Lune) and uninvaded streams (*n* = 2; Clow Beck and River Greta), obtained through Linear Mixed-Effects Modelling (LMM). Linear model summaries are available in Table S4.8.

WQ	Group	Stream	LMM results			
			Mean square	*df*	*F*	*P*
Dissolved oxygen	Invaded	Alwent	<0.001	1, 27.2	0.31	0.582
		Balder	<0.001	1, 24.1	0.17	0.687
		Deepdale	<0.001	1, 12.8	0.36	0.559
		Lune	0.001	1, 29.6	0.63	0.433
	Uninvaded	Clow	0.004	1, 19.3	1.72	0.205
		Greta	0.001	1, 22.2	1.02	0.324
BOD	Invaded	Alwent	0.037	1, 83.4	4.79	**0.031**
		Balder	0.037	1, 23.0	3.23	0.086
		Deepdale	0.054	1, 58.1	7.64	**0.008**
		Lune	0.067	1, 23.1	7.13	**0.014**

Table 4.13 Continued.

WQ	Group	Stream	LMM results			
			Mean square	*df*	*F*	*P*
BOD	Uninvaded	Clow	0.278	1, 19.8	13.47	**0.002**
		Greta	<0.001	1, 62.7	0.003	0.959
Hardness	Invaded	Alwent	0.007	1, 8.1	1.96	0.199
		Balder	0.015	1, 18.4	7.75	**0.012**
		Deepdale	0.063	1, 38.9	2.28	0.140
		Lune	0.007	1, 17.6	1.75	0.203
	Uninvaded	Clow	<0.001	1, 10.4	0.10	0.760
		Greta	0.109	1, 65.4	2.38	0.128
Nitrogen	Invaded	Alwent	0.007	1, 28.8	1.37	0.252
		Balder	0.002	1, 25.2	4.46	**0.045**
		Deepdale	0.001	1, 75.6	0.20	0.657
		Lune	0.003	1, 23.3	4.60	**0.043**
	Uninvaded	Clow	0.019	1, 23.1	5.86	**0.024**
		Greta	0.006	1, 11.0	1.26	0.285
Ammonia	Invaded	Alwent	0.0003	1, 127.9	4.38	**0.038**
		Balder	0.0003	1, 26.5	5.51	**0.027**
		Deepdale	<0.001	1, 18.7	0.24	0.628
		Lune	<0.001	1, 22.1	0.50	0.489
	Uninvaded	Clow	<0.001	1, 25.7	1.87	0.184
		Greta	<0.001	1, 14.3	3.22	0.094
pH	Invaded	Alwent	0.001	1, 29.8	6.66	**0.015**
		Balder	<0.001	1, 32.8	0.36	0.555
		Deepdale	0.0004	1, 16.8	2.55	0.129
		Lune	0.0003	1, 21.4	4.04	0.057
	Uninvaded	Clow	0.0002	1, 29.5	4.66	**0.039**
		Greta	<0.001	1, 25.1	0.87	0.361
Water temperature	Invaded	Alwent	0.004	1, 143.8	0.47	0.493
		Balder	0.003	1, 147.7	0.48	0.491
		Deepdale	0.011	1, 88.9	0.74	0.393
		Lune	0.001	1, 134.5	0.09	0.764
	Uninvaded	Clow	0.004	1, 130.5	0.42	0.519
		Greta	<0.001	1, 127.6	0.002	0.960
Turbidity	Invaded	Balder	0.303	1, 66.0	8.25	**0.005**
		Lune	0.332	1, 57.1	14.40	**<0.001**
Zinc	Invaded	Alwent	0.037	1, 21.4	1.70	0.206
		Balder	0.033	1, 27.7	2.74	0.109
		Deepdale	0.002	1, 62.0	0.06	0.808
		Lune	0.003	1, 129.3	0.42	0.520
	Uninvaded	Clow	0.016	1, 16.9	1.11	0.307
		Greta	0.012	1, 58.9	1.25	0.269

4.4 Discussion

This study provides evidence that native fish abundance and the community structure of fishes and benthic invertebrates are being strongly impacted by signal crayfish in streams of a typical English upland limestone river system. In particular, small benthic fishes such as bullhead and YoY trout declined in streams where signal crayfish became abundant, but those in uninvaded streams did not, while habitat did not change in invaded and uninvaded stream survey sites over the 7-year period. This confirms similarities between laboratory based study outcomes (Guan and Wiles, 1997; Edmonds *et al.*, 2011; Findlay *et al.*, 2015) and the situation in the wild (this study).

4.4.1 Population, and invasion, of signal crayfish in upland streams

The impacts of invaders may take time to become apparent in habitats, depending on the mode of action and whether impacts are density-related (Simberloff *et al.*, 2013). Few studies have presented historical timelines of changes in density of invasive crayfish following initial colonisation, but in this study it is evident that the density of signal crayfish increased in pre-invaded streams between 2011 and 2018. Tributaries are being invaded quite quickly, with three sites having no signal crayfish recorded in 2011 but present in 2018 (Lune, Sudburn and Thorsgill). Signal crayfish exhibit wide tolerance and activity physiologically (Lirås *et al.*, 1998; Bubb *et al.*, 2002; McMahon, 2002) and ecologically (Holdich, Rogers, *et al.*, 1999; Karjalainen *et al.*, 2015) as well as a strong dispersal propensity (Bubb *et al.*, 2006a), aiding rapid colonisation of habitats. In addition, rapid growth, early maturation and greater fecundity also make them a successful invader (Westman and Savolainen, 2001). Nevertheless, this study suggests that signal crayfish in the upper Tees are still very much in a population expansion phase; community impacts can therefore be expected to continue with time and are likely to become permanent.

Both size-distribution and sex ratio can influence signal crayfish invasion (e.g. Light, 2003; Wutz and Geist, 2013). A higher proportion of larger signal crayfish can strongly affect stream communities and habitats,

by increasing direct predation on fish (Guan and Wiles, 1997), and altering habitats through burrowing behaviour (Guan, 1994). Large signal crayfish are also capable of rapid, active upstream movements, facilitating colonisation (Bubb *et al.*, 2006a; Wutz and Geist, 2013). The ratio of females to males was slightly higher in pre-invaded Tees streams in 2011 (♂ : ♀, 1 : 1.12) and it increased to 1 : 1.29 (♂ : ♀) in 2018. However, deviation from the expected sex ratio of 1 : 1 indicates that the invasion by signal crayfish has not been completed yet (Capurro *et al.*, 2007).

4.4.2 Impact on benthic fishes and YoY salmonids

BIOENV analysis, based on 2011 vs. 2018 data revealed that abundance of signal crayfish was a key factor in shaping the fish communities in the invaded streams. Habitat factors were important too but signal crayfish abundance was included in all models explaining the highest levels of variation.

Small benthic fishes such as bullhead, a sculpin, were particularly vulnerable to signal crayfish invasion in the Tees. Signal crayfish can exclude benthic fishes from shelters and make them susceptible to predation (Rahel and Stein, 1988; Guan and Wiles, 1997) by crayfish predators such as brown trout (*S. trutta*), eel (*A. anguilla*), heron (*Ardea cinerea*), mink (*Neovison vison*) and otter (*Lutra lutra*) in the study streams. Bullhead seek shelter in refuges, such as under stones, during daylight (Mills and Mann, 1983), as do signal crayfish (Bubb *et al.*, 2009). Although large sculpins can eat or displace the smallest crayfish, in European streams the competitive interaction is heavily asymmetrical in favour of crayfish (Bubb *et al.*, 2009) and this was supported by the mesocosm experiments in Chapter Two. Disappearance of bullhead in two pre-invaded streams in this study could be associated with the high density of signal crayfish, as a great reduction in benthic fishes or even local extinctions are possible in habitats with a high density of signal crayfish (Guan and Wiles, 1997; Bubb *et al.*, 2009). The population decline in sculpins is likely partly due to increased egg mortality since males normally guard the eggs, but may be driven away by signal crayfish (M. Lucas, pers. comm.), and is likely partly due to increased predation risk to juveniles and adults leaving

shelter. Evidence supporting this mechanism of impact is provided by the significant differences in length-frequency distributions of bullhead between 2011 and 2018 (Figure 4.5). In uninvaded streams there was no difference in size (age) structure of bullhead with good recruitment of younger age groups into the population, but in pre-invaded streams there was a highly significant difference between 2011 and 2018, with negligible numbers of young and evidence of major recruitment failure.

These comments regarding impacts of crayfish on benthic fishes are reserved for fish species with small ultimate body size; larger benthic fishes such as European eel *Anguilla anguilla* can predate signal crayfish (Blake and Hart, 1995) and are unlikely to be displaced by crayfish, but eel were very rare at the study sites and occur only in low densities in the upper Tees, partly due to migration barriers further downstream.

Reduced densities of YoY salmonids occurred in Tees streams invaded prior to 2011, by comparison to 2018, but not in uninvaded (control) streams, demonstrating that this was not a universal recruitment failure effect but specific to invaded streams. The cause of reduced densities of YoY salmonids in crayfish-invaded streams was less clear-cut, since there was no statistical evidence of recruitment failure from length-frequency comparisons of invaded and uninvaded streams.

Reduced densities of YoY salmonids in crayfish-invaded streams may be a result of any of three signal crayfish mediated factors. Firstly, salmonid eggs and alevins may be predated by crayfish (Edmonds *et al.*, 2011; Findlay *et al.*, 2015), though Gladman *et al.* (2012) found no evidence of signal crayfish detecting and digging out buried eggs from artificial redds. Secondly, competition between salmonid fry and crayfish for shelter (Griffiths *et al.*, 2004). Lastly, an increase in fine sediment infiltration into spawning habitats due to zoogeomorphic processes involving crayfish (Nyström *et al.*, 1996; Harvey *et al.*, 2011; Mathers *et al.*, 2019) might reduce survival of salmonid eggs and alevins, which are sensitive to fine sediment (Harvey *et al.*, 2011). However, severe negative impacts on macroinvertebrates in invaded habitats, revealed through S_1–S_3 of this study, may also have played a key role for this decline. Salmonids prefer to

prey on different macrointvertebrate taxa including Trichoptera (caddis fly larvae) (Giller and Greenberg, 2015) and therefore significant reduction in the abundance of these taxa (e.g. Rhyacophilidae and Hydropsychidae) in invaded habitats may result in poor feeding considitions for salmonids. In addition to these causes, salmonid recruitment is impacted by poor water quality, poor habitat, migration barriers, and excessive fine sediment due to poor land management (Peay *et al.*, 2009). Interestingly, an increased number of non-YoY salmonids were recorded in invaded streams, possibly due to immigration from outside the survey zone, or due to increased survival resulting from reduced competition by YoY trout. Certainly older trout would be able to benefit from feeding on crayfish. This confirms that crayfish invasion may be beneficial for large individuals to some extent, as recorded for large chub (*Squalius cephalus*; Wood *et al.*, 2017).

At the sites in this study all of these habitat, environmental and water quality factors remained relatively constant over time and it seems unlikely they were responsible for the decline in YoY salmonid densities at pre-invaded sites by comparison to uinvaded sites. Physical obstacles are few between the main channel and the stream study sites in the upper catchment and habitat remained similar. Most water quality variables remained stable in invaded streams. Turbidity (which would increase with elevated concentrations of suspended solids) decreased markedly in one invaded stream but increased slightly in another stream (River Lune). It was not immediately evident what has caused this increased level of turbidity in the stream but it may be related to signal crayfish activities (e.g. foraging but not burrowing as no burrows were recorded) as higher level of turbidity was recorded after the establishment of crayfish population in the stream (since 2011). Nitrogen and ammonia decreased significantly.

4.4.3 Impact on macroinvertebrate communities

As hypothesised, strong impacts were recorded on macroinvertebrates in signal crayfish invaded streams. The first study (S_1; 2011 vs. 2018) results revealed significant and increasing deviation in macroinvertebrate communities between invaded and uninvaded streams over time. Differences in community structure as well as reduced taxonomic richness

and total invertebrate density occurred between invaded and uninvaded reaches of the same streams (S_2). Given that most temperate zone freshwater macroinvertebrates (other than crayfish and large bivalves) have a generation time of 1–3 years, and that by 2011, signal crayfish had already been in the pre-invaded streams for about a decade, this could already have generated a change in the community that differed from uninvaded streams in 2011. Mathers *et al.* (2016) showed that benthic macroinvertebrate communities typically took 5–10 years for major change to be evident following signal crayfish invasion. I did not find any time effect on the invertebrate community in newly-invaded streams, invaded for >5 years. However, negative impacts on taxonomic richness may take more time to become evident as no macroinvertebrate family changed significantly in newly-invaded streams (S_1) but changes were evident in streams invaded by crayfish for ~20 years (S_3).

The negative effects of signal crayfish on several invertebrate taxa (Nyström *et al.*, 1996; Nilsson *et al.*, 2012) have already been recognised. Crayfishes, including signal crayfish, can alter invertebrate community structures directly, primarily through predation, or indirectly via trophic cascades (Bondar *et al.*, 2005; Jackson *et al.*, 2014). However, similar to fish communities, macroinvertebrate data in S_1 (2011 vs. 2018 data) represent a limited temporal context.

Long-term (1990 – 2017) Tees data in the third study (S_3) show that despite similar taxonomic richness in macroinvertebrate communities between invaded and uninvaded sites before signal crayfish invasion it decreased significantly after the invasion. However, in streams invaded by crayfish, a shift towards more mobile invertebrate taxa adapted to high flow velocities could be expected at the expense of less mobile taxa (e.g. Mollusca, Hirudinea and case-bearing caddis flies; Parkyn *et al.*, 1997; Keller and Ruman, 1998; Wilson *et al.*, 2004; Dorn, 2013). The negative impacts of crayfish on molluscs are probably the most often reported (Weber and Lodge, 1990; Lodge *et al.*, 1994; Nyström *et al.*, 2001; Mathers *et al.*, 2016). In this study, the pulmonate snail family Ancylidae decreased significantly following signal crayfish invasion whereas an opposite trend was observed in uninvaded streams. The bivalve mollusc family

Sphaeriidae, decreased in invaded streams but increased in uninvaded streams. Abundance of both families was significantly higher in uninvaded sections compared to signal crayfish invaded sections within the same streams. This may primarily be due to direct consumptive effects by the crayfish (Wilson *et al.*, 2004; Dorn, 2013) as the limited locomotion of gastropods makes them very susceptible to crayfish predation (Hanson *et al.*, 1990; Rosewarne *et al.*, 2013).

The leech subclass Hirudinea is also considered one of the susceptible groups of invertebrates to crayfish invasion (Stenroth and Nyström, 2003; Crawford *et al.*, 2006; Ruokonen *et al.*, 2014). In this study, the leech family Glossiphoniidae declined in invaded streams whereas its occurrence increased in uninvaded streams. An opposite trend was recorded for another leech family Erpobdellidae. This may be due to the differences in reproduction (Mathers *et al.*, 2016) in which glossiphonids brood and carry their young and erpobdellids form cocoons on the substrate that may protect the young from predation (Elliott and Mann, 1979). However, all these groups are slow moving benthic invertebrates and a reduction in their abundance is in accordance with the hypothesis of this study.

The stonefly family Perlidae decreased in both stream categories but significantly in crayfish invaded streams. A reduced abundance of stonefly (Plecoptera) has been reported earlier from the crayfish invaded parts of the River Clyde in Scotland (Crawford *et al.*, 2006). However, several stonefly families are also predatory groups that could potentially compete with small crayfish and their abundance may be greater in places with no crayfish (Ruokonen *et al.*, 2014). Caddisflies exhibited increases or decreases in abundance depending on the families. Lepidostomatidae, Polycentropodidae and Rhyacophilidae reduced significantly following signal crayfish invasion but the latter two taxa also decreased in uninvaded streams. The opposite trend occurred for Glossosomatidae and Goeridae. This may be due to variation in external protection; the latter groups are case-bearing caddisfly and they make hemispherical portable cases entirely made of sand grains and silk (Cox and Wagner, 1989; Becker, 2001; Nijboer, 2004) that offer more protection against predators,

compared to Polycentropodidae and Rhyacophilidae, caseless caddisfly families.

The relative abundance of the amphipod family Gammaridae remained almost unchanged in invaded streams following crayfish invasion and increased slightly in uninvaded streams. No significant variation in abundance of this family was recorded between invaded and uninvaded parts of the same streams. This group is also reported to remain unchanged following signal crayfish invasion from lowland rivers of the UK (Mathers *et al.*, 2016). The adoption of various avoidance strategies by this group (e.g. enhanced drift and locomotion, vertical migration and increased use of refuges) enables them to successfully evade inter and intra-specific predation (Andersson *et al.*, 1986; McGrath *et al.*, 2007; Haddaway *et al.*, 2014). However, evidence of significant decrease in crustacean macroinvertebrates in invaded sections of streams is also available (Crawford *et al.*, 2006). Being an important part of food web, decrease in macroinvertebrates may negatively affect the ecosystem in many ways. Reduced macroinvertebrate densities could accelerate the deposition of organic matters (Wallace *et al.*, 1982; Appelberg *et al.*, 1993) and could potentially impact biology of other species.

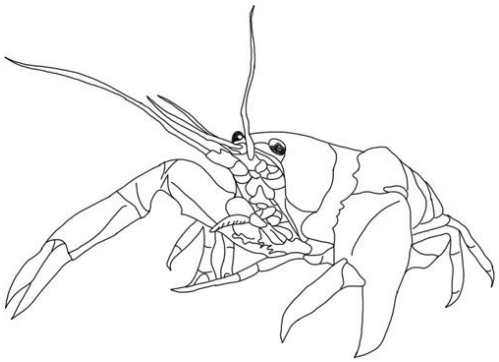
4.5 Conclusions

This study supports the hypothesis that native fishes, especially benthic and YoY salmonids, and less mobile macroinvertebrate taxa are declining because of the non-native signal crayfish invasion in upland English streams. This study shows that this is not due to habitat change and that water quality has remained good or, generally, improved and cannot be considered causal either. Impacts of non-native crayfish may not be evident immediately after colonisation, because of their slow invasion rate during establishment and more rapidly thereafter (Guan and Wiles, 1996; Peay and Rogers, 1999; Bubb *et al.*, 2005). Nevertheless, a considerable reduction in abundance of the recipient communities may be evident, as has happened for the newly-invaded streams in this study. This study also concludes that, in a signal crayfish invaded stream, the macroinvertebrate

community will be impacted before the fish community as significant changes were recorded in macroinvertebrates in streams invaded for >5 years (i.e. newly-invaded streams in S_1) but no such change in fishes was determined over the same timescale. In streams invaded by crayfish for >8 years negative effects on fishes were recorded in few cases but the macroinvertebrate community was found to be suffering from severe impacts.

Population characteristics (density, size-distribution and sex ratios) of the invading signal crayfish population in the upper Tees shows it is still expanding rapidly, although some stream subpopulations are probably now close to carrying capacity. This may pose a major threat to the native fish populations, particularly to the benthic bullhead and may result in local extinction of the species, as recorded in two pre-invaded streams of this study. Recruitment of trout populations in these rivers may also be strongly impacted as signal crayfish approach carrying capacity. The temporal pattern of fish and invertebrate impacts observed in this study supports the hypothesis that disruption of ecological conditions in signal crayfish invaded streams will be greatest as crayfish reach carrying capacity.

This study provides information on distribution, population density, age groups and sex ratios (for crayfish only) of signal crayfish and native fish populations. These information may help in determining an appropriate strategy for managing crayfish invasions in relation to fisheries and conservation (Moorhouse *et al.*, 2014) where, rather than stocking, natural salmonid fisheries are being encouraged (Peay *et al.*, 2009). The findings of this study suggest that widespread and long-term ecological disruption is occurring in upland streams and that preventing further introductions and spread of non-native crayfish is crucial to limiting the extent of those impacts.



Chapter Five

GENERAL DISCUSSION

This thesis aimed to determine the direct and indirect impacts of non-native signal crayfish *Pacifastacus leniusculus* on native upland stream biota and ecosystem processes. It also aimed to determine underlying mechanisms of biological invasion by an animal model species (signal crayfish) dispersing in the wild. Field surveys of a large number of upland streams revealed the population status of native fishes and crayfish in terms of their density, size groups and sex ratio (latter for crayfish only) in the River Tees, northeast England. It is expected that the outcomes of this thesis have contributed to the field of biological invasion and enhanced our knowledge in better understanding of impacts and factors affecting non-native crayfish invasion, which in turn, can play an important role in the management of introduced crayfish. Impacts of signal crayfish were evaluated on short- (~7 weeks), medium- (7 years) and long- (28 years) timescales (Chapter Two and Four) and key factors driving signal crayfish invasion (i.e. dispersal of established subpopulations) were determined in the wild (Chapter Three). In this chapter, a brief summary of the findings, in relation to thesis aims, is presented along with consideration of research limitations, management options, conclusions and recommendations for future research.

5.1 Summary

5.1.1 Impacts of crayfish on native biota and ecosystems

In the Anthropocene, assessment of the impacts of non-native species on native species and the environment is a priority (McNeely *et al.*, 2001; Richman *et al.*, 2015; Panlasigui *et al.*, 2018). Two studies of this thesis, described in Chapters Two and Four, revealed strong impacts of the signal crayfish on native biota and ecosystems and strongly supported the hypotheses (1, 2 and 4; section 1.7 in Chapter One) of the study that native benthic fish species, macroinvertebrates and important ecosystem processes would be negatively affected by non-native signal crayfish and result in loss of native species, shifts in the macroinvertebrate communities and alteration of the food web in the affected ecosystem.

It is often the case that all the potential factors are not considered at the time of evaluating impacts of a non-native species. Moreover, many existing studies of the impacts of invasive species, including crayfishes,

were conducted in controlled laboratory environments for a short period. Therefore the outcomes of short-term laboratory-based studies may not be appropriate in predicting impacts of a non-native species in natural environments due to the absence of numerous limiting factors in the laboratory (Degerman *et al.*, 2007). Therefore, in the first study (Chapter Two), impacts of different densities of signal crayfish on native benthic fish species and ecosystem processes were determined in the River Lune, using a classical mesocosm approach. The experiment was set within the river channel, therefore allowing the impacts to be determined under near-natural conditions. Results of this study showed strong effects of signal crayfish on native benthic bullhead, macroinvertebrates, leaf litter decomposition and algal growth. Therefore similar impacts may also be expected in other upland running water habitats invaded by signal crayfish. A complementary study described in Chapter Four showed medium– to long–term impacts of signal crayfish on fish and macroinvertebrate communities in streams of the River Tees, NE England. Ideally, such studies should be replicated on other upland rivers to determine the extent of general patterns, but such research is intensive and was not possible in this thesis. Nevertheless, it is believed that the impacts of non-native signal crayfish on indigenous biota in upland stream ecosystems are evident through the combined results of these two studies.

These studies showed that signal crayfish can negatively affect benthic bullhead growth, even at a low crayfish density (Chapter Two), reduce bullhead abundance and even lead to its complete disappearance (Chapter Four). Previous studies have provided evidence of aggressive behaviour towards bullhead and dominancy over this species in laboratory conditions (Bubb *et al.*, 2009; Findlay, 2013). In the field, a negative relationship was observed between the abundance of signal crayfish and bullhead in upland and lowland rivers (Guan and Wiles, 1997; Bubb *et al.*, 2009). Guan and Wiles (1997) showed evidence of direct predation of crayfish in mesocosm conditions. However, no previous study has shown impacts at various densities of crayfish on a benthic fish species like bullhead or before-after invasion comparisons in invaded habitats.

Strong negative effects of signal crayfish were also recorded on macroinvertebrate taxonomic richness, abundance and communities in both studies within this thesis. These outcomes are in accordance with several other studies (Nyström *et al.*, 2001; Crawford *et al.*, 2006; Mathers *et al.*, 2016).

Significant adverse effects of signal crayfish on leaf litter decomposition and algal growth were also recorded. These are critical ecosystem processes at the base of aquatic food webs and may play an important role in determining trophic cascades (Woodward *et al.*, 2008), although cascades were not evident in this study. It is evident from the current study that these changes were due to signal crayfish effects as all other important parameters (e.g. habitat conditions and native fish density) were similar across all enclosure groups. Although it was expected that dramatic reductions in shredders or predatory macroinvertebrates would lead to a trophic cascade, direct crayfish feeding on leaf matter appears to have been more important in this study in an upland stream ecosystem. This was also evident in the diet analysis of signal crayfish in which greater consumption of leaf litter can be assumed in higher density treatment groups.

Results of the medium- and long-term studies of signal crayfish impacts on native fish and macroinvertebrate communities in upland streams (Chapter Four) reflect enclosure-exclosure (the mesocosm) study outcomes, conducted in the River Lune. This is the first long-term study that reported impacts of signal crayfish on fishes, especially YoY salmonids and benthic fishes, and macroinvertebrates in upland rivers. By contrast, the study of Peay *et al.* (2009) was over a small spatial and temporal scale. In addition, in the current study, stream habitat and water quality were also recorded to better understand the changes. Surveys performed in 2011 and 2018 showed a significant decrease in abundance of native fishes in recent time in streams invaded by signal crayfish before 2011. These impacts were particularly evident on the abundance of benthic fishes (bullhead and stone loach) and YoY salmonids. On the other hand, in control streams without signal crayfish (i.e. uninvaded streams) abundance of these species or groups increased over the same time. Although the mesocosm

study (Chapter Two) showed competition effects on bullhead, the field results (Chapter Four) suggest population effects on bullhead occurred through recruitment failure. Given that bullhead lay eggs on the undersides of stones, consumption of these by crayfish in a density-dependent manner seems highly likely, but remains to be demonstrated.

The study described in Chapter Two (mesocosm or enclosure-exclosure) was limited by only investigating the impacts of signal crayfish belonging to a specific size group (25–30 mm CL). Further research which investigates the impacts of signal crayfish outside the size range considered in this study is recommended. Similarly, this study focused on a specific size class of bullhead and there is room for further research on remaining size classes. The study described in Chapter Four involved two samples in 7 years (in 2011 and 2018) and was therefore a limitation of the study (i.e. provides limited temporal context). However, a large sample size (n = 18 streams) is believed to have helped minimise this limitation by revealing a range of colonisation scenarios over a broader area. Moreover, findings of this study (Chapter Four) were also in agreement with the mesocosm experiment in the River Lune (Chapter Two) and thus it can be expected that, despite limited temporal contexts, the study results sufficiently revealed the impacts of signal crayfish on native fishes.

One of the major indirect effects of signal crayfish and other invasive crayfishes is as ecosystem engineers, particularly through their burrowing actions and by their processing of sediment (Harvey *et al.*, 2011; Rice *et al.*, 2012; Albertson and Daniels, 2016). This effect was not part of the current study. It is known that burrowing and other sediment alterations by crayfish can be major effects in certain aquatic habitats (see section 1.5.1), particularly lowland streams. In the study streams of this thesis burrowing was relatively unimportant as less burrowing was recorded in surveyed streams, probably due to the availability of suitable in-stream refuges (e.g. unembedded boulders and cobbles) for crayfish. But, it may be important in stretches with a low amount of suitable in-stream refuge availability, which may be a more common feature of lowland rivers. Burrowing is also dependent on bank soil structure; crayfish burrow readily in clay and other densely-packed soils but not in sandy soils (Guan, 1994).

Burrowing activities of crayfish result in an increased amount of fine sediment mobilisation into the aquatic habitat which may impact on several components of the ecosystem, both directly or indirectly. This process can affect biology (growth, reproduction, mortality etc.) at all trophic levels (Henley *et al.*, 2000) and can be responsible for reductions of macroinvertebrates (Larsen and Ormerod, 2010; Jones *et al.*, 2012; Mathers *et al.*, 2017) and fishes (Kemp *et al.*, 2011; Reaney *et al.*, 2011) as a result of reduced trophic resources, infilling of streambed shelters and altered ecosystem functioning.

Although the upland streams studied in this thesis differed in geomorphology and hydrological characteristics from lowland rivers and lakes in many crayfish studies, the impacts of signal crayfish on macroinvertebrates and fish recorded in this thesis were quite similar to those reported in lowland rivers (Guan and Wiles, 1997; Mathers *et al.*, 2016) and closed water bodies including ponds and lakes (Nyström *et al.*, 1996; Ruokonen *et al.*, 2014). This reflects a strong capability of invasive crayfishes such as signal crayfish to affect a wide range of freshwater ecosystems, provided that they are not strongly acidic, have sufficient calcium levels and offer suitable physical habitat. It should be noted that upland streams are quite different to some other stream habitats in several regards and this may influence the ways by which signal crayfish exert effects. For example, in lowland chalk, primary productivity is dominated by in-stream macrophytes, especially large beds of water crowfoot (*Ranunculus* spp.), rather than the epilithic algae typical of upland streams. In chalkstreams the hydrological regime is relatively stable, the average sediment particle size is also smaller (gravel mostly) and crayfish burrow more at the base of banks and mobilise fine sediment. However, frequent high-flow events and relatively higher flow velocity in higher-gradient upland streams may also result in less sediment deposition in upland habitats. Recent research (Rice *et al.*, 2019) suggests that the feeding behaviour of benthic fishes including bullhead may impact bed materials in lowland rivers. However, this is unlikely in upland rivers with large sediment sizes and bedrock outcrops, including those in the current study, where

fishes with strong zoogeomorphic capability (e.g. large benthic cyprinids) do not exist.

5.1.2 Drivers of invasive crayfish dispersal

Chapter Three of this thesis identified the factors that influence signal crayfish dispersal, particularly in an upstream direction, in stream ecosystems and thus contributed important knowledge to understand invasion dynamics of an important non-native species. Findings of this study also support the hypothesis that bold individuals are responsible for initiating and maintaining an 'invasion front' (hypothesis 3 from section 1.7). However, this study also revealed that crayfish dispersal in natural conditions is not only affected by its personality traits but also by habitat characteristics and the local population density of crayfish. There are relatively few studies on personality-dependent dispersal for invasive animals, including aquatic ones (Duckworth and Badyaev, 2007; Cote, Fogarty, *et al.*, 2010; Malange *et al.*, 2016) (see section 3.1 for details). However, many studies have demonstrated the importance of factors such as propagule pressure and the suitability of recipient habitats in species invasion (Lockwood *et al.*, 2005; Simberloff, 2009a; Warren *et al.*, 2012). Others have examined the role of personality in animal invasion (Duckworth and Badyaev, 2007; Cote *et al.*, 2011; Cucherousset *et al.*, 2013) but few have combined these ecological and behavioural factors. Laboratory-based personality studies of dispersal and invasive species may not be directly applicable in natural conditions.

The study described in Chapter Three, conducted at fully-established, newly-established and invasion front sites, confirmed that personality traits can be highly consistent over time in signal crayfish and correlated to each other and, therefore, form behavioural syndromes. Contrary to some other personality studies (e.g. Cote, Clobert, *et al.*, 2010), the activity trait was negatively related to boldness and exploration traits of signal crayfish in this study. The key personality traits driving the dispersal of invasive crayfish were found to be boldness, exploration and climbing. However, roles of these traits in dispersal of crayfish may vary across sites; dispersal was negatively associated with boldness, exploration and

climbing at a site where signal crayfish were fully-established, but dispersal was positively associated with these traits at newly-established and invasion front sites. This is believed to be the first study that has shown a behavioural trait can influence dispersal both positively and negatively, depending on contexts. Previous studies did not consider a trait's role in dispersal under different circumstances and therefore, it was not possible to observe different roles of a personality trait in the wild.

For some animal groups like decapods, climbing is an important personality trait to consider with regard to dispersal or invasion tendency. This is the first study (Chapter Three) with aquatic species in which climbing was evaluated as a potential driver of dispersal. This study found that, at the individual level, climbing tendency plays a significant role in the dispersal of non-native signal crayfish. Crayfish movement upstream is known to be affected by in-stream barriers, both large and small (Light, 2003; Kerby *et al.*, 2005; Bubb *et al.*, 2006b, 2009). In an upland stream, it is quite often the case that, even if there is no artificial barrier, there will be natural barriers that can prevent animal dispersal (Alò and Turner, 2005; Hansen *et al.*, 2014; Lennox *et al.*, 2019). Therefore, it is not a surprising outcome that crayfish with high climbing ability would lead the invasion process, as revealed in this study.

In addition to personality traits, in sites with a higher crayfish density (i.e. fully-established and newly-established) the availability of refuges can also play a significant positive role in dispersal tendency. It is expected that refuge availability is important for the species that require shelter, such as crayfish which shelter by day and are mostly nocturnal (Bubb *et al.*, 2009). When the population density is low (i.e. low-competition environment), a high proportion of refuges may not be occupied, and so refuge availability may not be an important consideration for crayfish behaviour. Thus, an influence of refuge availability at the (low-crayfish-density) invasion front may not be expected, as revealed in this study. By contrast, to find a suitable shelter, dispersal over a longer distance may be expected within any high-density population of crayfish or, more particularly, conditions where the ratio of refuges to crayfish numbers is low. Under such

conditions intraspecific competition is likely to be the key mechanism at play.

In this study, in Thorsgill Beck, the distance between newly-invaded and invasion front site centres was ~500 m which is quite a short distance. Marked crayfish could have moved between those sections, as this species is capable of long-distance movement within short period (e.g. 341 m in two days in an upland stream with ~20 m channel width; Bubb *et al.*, 2006a). But, in both streams (Westholme and Thorsgill) signal crayfish did not disperse more than 125 m, at a rate of less than 5 m day^{-1} which indicates that in a stream of comparatively smaller width (~5 m) movement rates of signal crayfish may be much slower than those in the main river (Bubb, 2004). This variation may be due to differences in physico-chemical factors, distribution of resources, prey-predator intecractions, refuge availability etc. between habitat types.

5.2 The outlook for British upland stream systems and native biota as non-native crayfish spread

This thesis has revealed rapid colonisation, strong impacts and factors driving invasive crayfish invasion in English upland streams and the findings of this thesis are believed to contribute to our understanding regarding crayfish invasion in running water systems. It is obvious that, if crayfish invasion continues without any effective control measures, many uninvaded upland habitats will be invaded.

The upland streams ecosystems are predicted to be less vulnerable to non-native crayfish invasion than lowland streams. This is partly because the lowland streams are low gradient and so have fewer impediments to upstream dispersal of invasive crayfish. Moreover, many lowland streams have been straightened and modified by humans and are inherently more stressed habitats which makes them more susceptible to biological invasion (Kennard *et al.*, 2005). On the other hand, streams in higher altitude areas can have more natural barriers (e.g. waterfalls) which make upstream spread of a non-native species difficult (Bubb *et al.*, 2006b,

2009). Invasive crayfish can alter habitats both directly and indirectly through competition for food and shelters, predation, physical disturbances (e.g. through burrowing and affecting sediment flux) as well as via trophic cascade (see section 1.5). These are the key factors that can affect biological communities in a habitat (Brewer, 1988). Some of the impacts in upland stream ecosystems have already been revealed in the study streams through this study (Chapter Two and Four) and by others (Bubb *et al.*, 2005, 2009; Peay *et al.*, 2009).

The potentially severe impacts in upland streams are revealed through complete disappearance of benthic fishes in two upland streams, invaded by signal crayfish for about 20 years, and marked reduction in abundance of benthic fishes in other invaded streams (Chapter Four). Although there is little evidence of species extirpation in the UK due to non-natives (Manchester and Bullock, 2000) this study provides evidence of local extirpation of benthic bullhead. More evidence is needed in this regard and thus, this issue demands more research. The white-clawed crayfish (WCC) species complex, the only native crayfish species to the UK and occurring across other parts of Europe including France and Ireland, is already known to be vulnerable to signal crayfish invasion. A range of studies (Bubb *et al.*, 2009; Peay *et al.*, 2009; Vaeßen and Hollert, 2015) have revealed adverse impacts of signal crayfish on the WCC and it is now one of the major causes of WCC decline, primarily due to its superiority over WCC and due to crayfish plague carried by signal crayfish (Holdich, 2003b). However, the WCC is now a globally endangered (Füreder *et al.*, 2010) species, protected in Europe under the Bern Convention and through the EU Habitats and Species Directive. In the UK it is also a protected species under Schedule 5 of the Wildlife and Countryside Act 1981 which makes it illegal to disturb it without license. But existing WCC habitats are at stake due to non-native crayfish in the wild and other human impacts such as pollution and habitat degradation (Peay, 2003; Peay *et al.*, 2009). White-clawed crayfish were formerly widespread in the River Tees prior to the introduction of signal crayfish, but were already in strong decline before signal crayfish spread widely (see section 4.2.1 for details). Just a few pockets of WCC remain in the Tees, for example in a small tributary of the

Balder, upstream of a waterfall, where signal crayfish have not yet colonised. Such remnant populations are at risk of extirpation due to stochastic events such as floods, disease or pollution. Efforts are being made by the Tees Rivers Trust to safeguard this and try to develop “Ark” sites locally (B. Lamb, pers. comm.). Although the “Ark” concept is established for WCC (Kindemba *et al.*, 2009; Nightingale *et al.*, 2017; Rosewarne *et al.*, 2017), its application for WCC conservation remains in progress. With signal crayfish widespread in the Tees, attempting to save WCC there is a questionable conservation objective. Instead, funding and effort would be better spent on safeguarding dense WCC populations in high-quality habitat catchments that have not been invaded by non-native crayfish – one such example is the River Wansbeck, Northumberland (Ream, 2010; Louca *et al.*, 2014). Yet that catchment is adjacent to the River Blyth (to the south) where signal crayfish are well-established and the risk of transfer to the Wansbeck remains high.

Although signal crayfish is the most widespread non-native crayfish in the UK as well as in Europe (Bubb *et al.*, 2004) many signal crayfish invaded habitats are also invaded by one or more other non-natives (e.g. Chinese mitten crab *Eriocheir sinensis* and other non-native crayfish species) (Jackson and Grey, 2013; Jackson *et al.*, 2014; Robinson *et al.*, 2019). Interactions among these non-natives are important in shaping the ecosystem structure and functioning (Jackson *et al.*, 2014; Rosewarne *et al.*, 2016). Studies in lowland habitats (the Thames catchment and ponds) revealed that the impacts of multiple non-native crayfishes on invaded ecosystems do not follow a general pattern and effects can be either additive or amplified (Jackson *et al.*, 2014). Bullhead, the native fish species in this study, are non-native in Scotland and spreading through salmon rivers like the Tweed and the Clyde (McLeish *et al.*, 2020) and may pose threats to local ecosystems. Non-native signal crayfish is also present in the Clyde and the Till, a Tweed tributary. Sculpins, such as bullhead, are known to predate salmon and trout eggs and may have significant impacts on some salmonid species (Findlay *et al.*, 2015). Given that this thesis shows signal crayfish have a strong negative impact on bullhead in upland streams, they might offset the impacts of invading bullhead on salmonids,

though the crayfish impacts could be major also. Thus further studies are needed to better understand the impacts on native ecosystems and interactions among multiple invaders in order to design an effective management programme.

It is obvious that bottom dwellers are most vulnerable to crayfish invasion. Apart from macroinvertebrates, species using benthic habitats for all their life (e.g. bullhead and white-clawed crayfish) are most at risk in invaded habitats. However, salmonid populations are also at stake in upland streams as they use riffle habitats as breeding and nursery grounds where non-native crayfish can also occur, which pose a threat to their recruitment. In addition to laboratory experiments on predation of salmonid eggs (Findlay *et al.*, 2015) this study revealed decreased YoY salmonid abundance in upland streams. Salmonids are economically important species, including for angling, and Atlantic salmon are regarded as threatened with extinction (Freyhof, 2014). Thus, the spread of non-native species, including signal crayfish is an additional stressor to existing major causes of decline such as migration barriers, flow regulation, habitat degradation, pollution, climate change and overexploitation (WWF, 2001; Forseth *et al.*, 2017; OECD, 2017).

As mentioned earlier in Chapter One (section 1.2) non-natives are an important driver of ecosystem change. Other anthropogenic factors (e.g. pollution and habitat degradation) are also contributing to this change (Dudgeon *et al.*, 2006; Suski and Cooke, 2007). However, in general or at least for the UK, non-native crayfish may pose greater threats to biodiversity protection over others as priorities have been given to improve or restore aquatic habitats via improving channel connectivity, water quality and others by the organisations concerned (e.g. Environment Agency; Sun *et al.*, 2020). On the other hand no single control measure has been found successful for controlling crayfish invasion (see below, section 5.3). Therefore, in future decades it is possible that crayfish invasion poses a greater threat to native biota and ecosystems than some existing stressors.

In the UK few non-native control or eradication measures have been successful (Manchester and Bullock, 2000) which indicates a need for

further actions. However, to ease the problems of the spread of non-native species, strengthening legislative provisions (see below for more, section 5.3) are suggested (Manchester and Bullock, 2000; Gherardi, Aquiloni, *et al.*, 2011; Boon *et al.*, 2020). Unfortunately, despite having additional international commitments to regulate non-native species (Bean *et al.*, 2006) legislative provisions to control non-natives are insufficient in the UK and it needs to be updated by enforcing and rewriting to improve the effectiveness (Manchester and Bullock, 2000). Moreover, public attitude and perception toward the non-native species in the UK do not correspond to greater ecological threats (Gozlan *et al.*, 2013; Williams *et al.*, 2019). For example, non-native common wall lizards (*Podarcis muralis*) in the UK show high levels of interaction with humans but UK people have low levels of knowledge regarding potential negative ecological impacts of this species (Williams *et al.*, 2019).

A case-by-case strategy for assessing risk has been recommended before introduction (Manchester and Bullock, 2000). For non-native crayfishes, already recognised as one of the top-ranked invasives taxa, strict restrictions have already been applied to prohibit their further spread. This includes restrictions on: returning a live crayfish without license, stocking them in the wild, keeping a live crayfish, and fishing for signal crayfish in sensitive geographical areas (DEFRA, 2019). However, continuous monitoring of non-natives in invaded ecosystems and development or enrichment of non-native species databases at regional, national and global scale are very much needed to prevent and manage biological invasions in the immediate future (Pimm *et al.*, 2014; Gallardo *et al.*, 2015).

5.3 Invasive crayfish management

Management of non-native species including non-native crayfishes to stop or reduce biological invasions is a global concern (Pimentel *et al.*, 2001; Luque *et al.*, 2014). To control, manage, and understand the spread of alien species an integrated approach is urgently needed (van Kleunen *et al.*, 2015). In order to establish a proper management strategy for invasive

crayfish in the UK and more widely, a method of controlling their further spread needs to be devised (Stebbing *et al.*, 2014). An effective solution to biological invasion, including invasion by crayfish, requires appropriate policies to address this, along with coordination between organisations dealing with the environment, health, trade, education etc. (Perrings *et al.*, 2009). It has been suggested that the intentional or accidental introduction of potential invaders should strictly be prevented and contingency plans should be designed to mitigate negative impacts (Manchester and Bullock, 2000; Gherardi, Aquiloni, *et al.*, 2011; Boon *et al.*, 2020). A three-stage hierarchical approach to management of alien invasive species has been suggested by the Convention on Biological Diversity (CBD) and these are (i) prevention, (ii) surveillance and rapid response, and (iii) control and eradication (Gherardi, Aquiloni, *et al.*, 2011; Roy *et al.*, 2014).

The CBD approach is complemented by the European Strategy that also includes raising awareness and dissemination of information on invasive species; strengthening national and regional capacity to deal with non-natives; and recovering native species and restoration of invaded habitats or ecosystems (Genovesi and Shine, 2004). The combined approach of the CBD and European Strategy is considered an excellent framework to follow in this regard (Gherardi, Aquiloni, *et al.*, 2011). However, implementing effective steps to prevent the dispersal and establishment of invasive species is a challenge to both conservation and international commerce (Mack *et al.*, 2000) due to a lack of harmony in existing regulations. For example European Council Regulation No. 708/07 to minimise risks of intentional introductions which has been in force in the European Union since 2009 has not yet controlled illegal or accidental introductions of non-natives (Gherardi, Aquiloni, *et al.*, 2011). In addition, The Global Invasive Species Programme (GISP) has also published a Guide for Designing Legal and Institutional frameworks on invasive alien species (IAS) with a view to supporting efforts to manage IAS nationally and internationally through a legal framework (Shine *et al.*, 2000; McNeely *et al.*, 2001). For this purpose a range of legal principles, approaches and tools (e.g. precaution, prevention, licencing, cost recovery, public participation and access to information, risk analysis, and impact

assessment) have been developed (Shine *et al.*, 2000). Increasing awareness of the impacts of non-native species through education programmes would be of great help in managing invasive species (Horwitz, 1990; IUCN, 2018; Manfrin *et al.*, 2019).

For invasive crayfishes, the population density can be extremely high and thus its control may be an extremely difficult job (Moorhouse and Macdonald, 2011). Rainfall can affect the distribution of invasive crayfish which sometimes might be relevant to controlling their distribution (Díaz-Paniagua *et al.*, 2014) but rainfall is a natural phenomenon and thus cannot be a solution. It has been suggested that high flow can play a vital role controlling signal crayfish invasion (Mathers *et al.*, 2020). However, for invasive crayfish, various methods of control have already been studied with advantages and disadvantages apparent for all (a summary of these methods are presented in Table 5.1).

As for other unwanted species, biological control methods have been considered for invasive crayfish. Otter (*Lutra lutra*) was found to be predatory on introduced red swamp crayfish *P. clarkii* in Portugal, especially during April to October (Beja, 1996) and they are known to prey heavily on native and non-native crayfish in the UK (Chanin, 2003; Almeida *et al.*, 2012), but are unlikely to prevent spread or colonisation. The performances of European eel (*Anguilla anguilla*) as a predator of non-native crayfish *P. clarkii* in the UK and Italy has been evaluated. They prey effectively on small crayfish and soft-shell crayfish and may be used as a compliment to traditional trapping method (Blake and Hart, 1995; Aquiloni *et al.*, 2010). Eels are nocturnal and so are active when crayfish are, making them more readily available than to diurnal predators. Eels can be abundant in upland streams if there is good stream connectivity and plenty of refuge habitat (boulder cavities, bank overhangs, crevices, tree roots), but in the current study (Chapter 4) very few eels were caught in the Tees due to large numbers of artificial barriers and the decline in the European eel population (Feunteun, 2002; Correia *et al.*, 2018). In another study, largemouth bass (*Micropterus salmoides*) proved to impose high predation risks to invasive crayfish (Gherardi, Mavuti, *et al.*, 2011) and thus it could be used as a potential predator, although largemouth bass are,

themselves, notorious invasive fishes throughout the warmer regions of Europe. Currently the UK is too cold for successful largemouth bass reproduction. Introducing predatory fishes may reduce crayfish survival, and hence population growth, but desirable impacts may not be expected soon after introduction (Holdich, Gydemo, *et al.*, 1999).

TABLE 5.1 Approaches considered for controlling crayfish invasion with major pros and cons, adopted and modified from (Gherardi, Aquiloni, *et al.*, 2011).

Categories & methods	Major pros (+) and cons (–)	References
Mechanical		
Trapping	+ Suitable for dense population, low ecological damage, catches only larger, older crayfish and so may reduce cannibalism – Costly, time consuming, low species-specificity, not effective in shallow areas	Hein *et al.* (2006); Moorhouse and Macdonald (2011); Nunes *et al.* (2017); Green *et al.* (2018)
Netting	+ May be effective for capturing small crayfish in streams – Labour intensive, requires kicking of substrates in streams, high environmental damages	Rogers and Holdich (1998); Holdich, Gydemo, *et al.* (1999); Sibley (2000)
Electrofishing	+ Low cost, fast method and low ecological damage – Low efficacy	Westman *et al.*, (1978); Sinclair and Ribbens (1999); Peay *et al.* (2015)
By hand	+ Low cost, high species-specificity and selectivity – Time consuming, difficult to catch smaller ones, low efficacy	Gherardi, Aquiloni, *et al.* (2011)
Physical		
Barriers	+ Once deployed minimum money and time are needed – Costly, low selectivity, potentially moderate impact on environment, no appropriate design for an effective barrier so far	Johnsen *et al.* (2008); Dana *et al.* (2011)
Drainage	+ Moderately time consuming – Low species-specificity, high cost involvement, high environmental damage, low efficacy unless dry for a long period	Holdich and Reeve (1991); Perrow *et al.* (2007)

TABLE 5.1: Continued.

Categories & methods	Major pros (+) and cons (–)	References
Diversion of rivers	+ Moderately time consuming – High cost involvement and impacts on environment, low species-specificity and low efficacy unless dry for a long period and combined with direct removal	Gherardi, Aquiloni, *et al.* (2011)
Biological		
Predation	+ Suitable for dense population, high selectivity and low / no environmental damage – Requires long period	Beja (1996); Aquiloni *et al.* (2010)
Pathogen	+ High species-specificity, low cost and time requirements and potentially high efficacy – Low selectivity in some cases, risk of unintended cross-species transmission	Diéguez-Uribeondo and Muzquiz (2005); Freeman *et al.* (2010); Gherardi, Aquiloni, *et al.* (2011)
Biocides		
Chemical	+ Less time consuming than mechanical means, high efficacy – Low species-specificity and selectivity for existing biocides, high environmental damage	Sandodden and Johnsen (2010)
Natural	+ Less time consuming, high efficacy – Low species-specificity and selectivity	Eversole and Seller (1997); Peay *et al.* (2006, 2019)
Autocidal		
Hormone	+ High species-specificity and selectivity, less environmental damages, low cost potentially – Requires longer time duration, low efficacy	Stebbing *et al.* (2003, 2004); Aquiloni and Gherardi (2010)
Sterile Male Release Technique (SMRT)	+ High species-specificity, low environmental damage – Not suitable for large population, low selectivity, required longer time, unknown efficacy	Aquiloni *et al.* (2009)

A major limitation of controlling crayfish with fish predators is that large crayfish are usually immune to predation by these predators, unless they are soft-bodied (e.g. Aquiloni *et al.*, 2010) and predation rate may be too slow to control fast-growing population of crayfish. For example,

European eel generally consume only about one crayfish every four days, perhaps due to their slow metabolism (Owen, 2001), which is unlikely to adequately control an established population of invasive crayfish.

Alteration of habitats has been discussed as a potential method to eradicate invasive crayfish populations and this can be done in several ways including temporary destruction of habitats and removing crayfish refugees (Freeman *et al.*, 2010). But, this method is not a practical solution to the problem in most running waters and stillwaters (Peay and Hiley, 2001) and involves high costs and severe environmental damage (Freeman *et al.*, 2010).

Application of chemicals to control signal crayfish population has been considered (Sandodden and Johnsen, 2010). However, use of toxic chemicals is not necessarily a good option for the environment, unless a highly specific, non-persistent pesticide is available. It is expected that the control method should be environment friendly. Biocides (e.g. pyrethroids), if used properly, may be the only solution, in some instances, to eradicate non-native populations (Simberloff, 2009b; Gherardi, Aquiloni, *et al.*, 2011). However, these are not particularly selective as, pyrethroids, for example, are notoriously toxic to a wide range of aquatic invertebrate species (Maund *et al.*, 1998; Rasmussen *et al.*, 2008, 2013; Nørum *et al.*, 2010). A considerable number of efforts have been undertaken to eradicate non-native signal crayfish populations in the UK, Norway and Sweden using biocides, with 50–100% success (in terms of crayfish mortality achieved) across several cases (Peay *et al.*, 2019). It is recommended that application of biocides should be carried out within two years of crayfish detection, preferably within one year, to achieve best eradication results (for details, see Peay *et al.*, 2019). Biocides may be an effective option for eradicating non-native crayfish population in small closed waters, especially in ponds, but may not be an option for running waters as it will also negatively impact non-target biota (e.g. native biota) and it may be difficult to prevent biocide dispersal downstream. Nonetheless, this may be possible in an isolated stream section or after flow diversion but its feasibility needs to be considered before application. However, there are examples of treating whole rivers with biocides (e.g. rotenone) in Norway to

eradicate the invasive alien ectoparasite *Gyrodactylus salaris* along with its host Atlantic salmon in the rivers to save the salmon population as well as local fishing tourism, recreation and business (Sandodden *et al.*, 2018). This works because *Gyrodactylus* cannot survive at sea, and a portion of the salmon population remains at sea and can then repopulate the river.

An autocidal method called the Sterile Male Release Technique (SMRT) has also been considered for controlling non-native crayfish populations. This method involves capturing or rearing crayfish followed by sterilizing and releasing large numbers of males into the wild to mate females with an aim to produce non-viable eggs (Aquiloni *et al.*, 2009; Gherardi, Aquiloni, *et al.*, 2011). However, the male individuals subjected to this method may not be competent enough to find and mate a female in the presence of wild males. Therefore its success is far from guaranteed. Males are also at a risk of shorter lifespan (Lance *et al.*, 2000; Lux *et al.*, 2002). To date the main sterilisation method attempted in the UK has been the low-technology approach of physical removal of the male gonopods but to date there are no published data on the effectiveness of such approaches. For pest insects and non-native Great Lakes sea lamprey (*Petromyzon marinus*) mass releases of sterile males have been achieved, but at high cost, and with limited success (Twohey *et al.*, 2003; Bergstedt and Twohey, 2005; Klassen and Curtis, 2005). Sex pheromone trapping attempts has also been made to control invasive signal crayfish (Stebbing *et al.*, 2003) but this is very much in its infancy and also, this method is not effective against both sexes of crayfish population (Aquiloni and Gherardi, 2010).

Research has shown that crayfish movement at night can be changed if they are exposed to artificial light (Thomas *et al.*, 2016) which might be used as an additional measure to control crayfish movement integrated with some other measures like physical restriction of the channel through which they move. Further research is warranted in this regard. It has been also revealed that crayfish movement and distribution is affected by path gradient, flow variation (Peay and Rogers, 1999; Light, 2003) and bed (bottom) materials (Johnson *et al.*, 2010; Louca *et al.*, 2014). These factors may help to control the spread of signal crayfish or other invasive

crayfish in natural habitats and could be very helpful to limit the movement and dispersal of invasive crayfish. Bubb *et al.* (2006b and 2009) have shown in streams that small vertical steps (a small natural waterfall and a small weir) can limit upstream movement of signal crayfish and white-clawed crayfish respectively. Artificial structures like large dams can prevent the spread of invasive crayfish (Kerby *et al.*, 2005). Several crayfish barriers have been installed to prevent invasion of invasive crayfishes in at least four European rivers with questionable effectiveness. One of these barriers is in the River Buåa at the Norway–Sweden border and invasive signal crayfish managed to bypass it (Johnsen *et al.*, 2008; Gherardi, Aquiloni, *et al.*, 2011). Three vertical barriers (1.5–2 m high) have been built in the mountain streams of southern Spain to prevent red-clawed crayfish *Procambarus clarkii* invasion which successfully achieved the goal but negatively affected the other biota as well (Dana *et al.*, 2011). In the UK crayfish barriers have been constructed in the River Clyde in Scotland (two barriers at a single location; Rahel, 2013) and the River Pont, England (two barriers at separate locations). In this PhD it was intended to examine the efficacy of the Pont barriers, but the installation locations (well upstream of the invasion front) by Northumberland Rivers Trust were not suitable to allow this. The Pont barriers were damaged severely by high flows within three months of deployment and the barrier efficiency in the Clyde remains unknown due to a lack of systematic study.

Apart from the field, a study conducted in the laboratory under controlled flow conditions identified potential barrier designs to limit upstream passage of crayfish while allowing passage of some fishes (Frings *et al.*, 2013). A recent study on selective passage of a barrier to exclude invasive crayfish showed that high flow velocity (2.39 m s^{-1}) can prevent signal crayfish from reaching the barrier base (Kerr *et al.*, 2020) but this flow velocity is too high and not common in the wild. However, this thesis shows that the climbing ability of crayfish is an important consideration in this regard and it may be possible to improve the effectiveness of the selective passage barrier by incorporating the climbing ability in the barrier design. This technique could be supplemented by an artificial by-pass channel near the barrier that crayfish would enter due to

their movement being blocked by the barrier. The bypass would have riffle habitat and suitable removable refuges could be constructed which would be subjected to regular control measures (removing and killing the crayfish within). However, it has been observed that the signal crayfish is capable of climbing over a 3-m high vertical barrier with coarse or rough surface (S. Rice, pers. comm.). Therefore, there is a clear need for more research efforts on the locomotory capabilities and motivations of invasive crayfishes, in order to develop effective control methods for them (Krieg and Zenker, 2020), especially on the design of an effective barrier surface (e.g. smooth), suitable gradients and materials, with consideration of the water flow types in the habitats occupied.

The outcomes of this thesis may be of help in managing signal crayfish invasion in the study area and elsewhere. Results of this thesis show that signal crayfish dispersal is strongly related to its personality traits, refuge availability and population density and the local population density is positively related to refuge availability (Chapter Three). A potential strategy to control signal crayfish spread may involve modification of a stretch of the stream of reasonable length (~500 m, i.e. multiple times the typical annual dispersal distance) upstream of the invasion front (if invasion is progressing towards upstream or vice-versa) by lowering or removing suitable in-stream refuge and supporting native predators (e.g. brown trout, eel and otter) in that section. The lack of suitable refuge will slow down or halt crayfish dispersal as well as expose crayfish to predators. However, the lack of refuge-size substrate may also impact predators such as trout and eel (Enefalk and Bergman, 2016; Degerman *et al.*, 2019; Enefalk *et al.*, 2019), so field testing of this strategy is needed. If supported, by experiments, this option may also be considered in streams with fully established populations of signal crayfish. In addition, as bigger crayfish are less susceptible to natural predators, mechanical control measures like trapping may be considered. Trapping is highly biased towards catching of larger crayfish (Wutz and Geist, 2013) and thus a combination of these methods might be effective in controlling crayfish invasion. Because trapping selects larger crayfish, it reduces cannibalism and so can increase survival of young crayfish, hence maintaining high

predation of young is crucial to buffering the potential for population rebound (Bills and Marking, 1988; Freeman *et al.*, 2010; Gladman, 2012). However, regular trapping may require huge labour and money which must be considered during the management planning. For the study area, this thesis also shows the availability of native fish species, including natural predators of signal crayfish (e.g. brown trout and eel) which may be of help in developing effective regional management strategies through the identification of (1) priority conservation sites (i.e. site with rich native species or high value species or low crayfish density), and (2) local sources with natural predators of crayfish to be used as a potential source of predatory species for biological control. The latter option may also be of help to cut down some of the management costs.

5.4 Concluding remarks

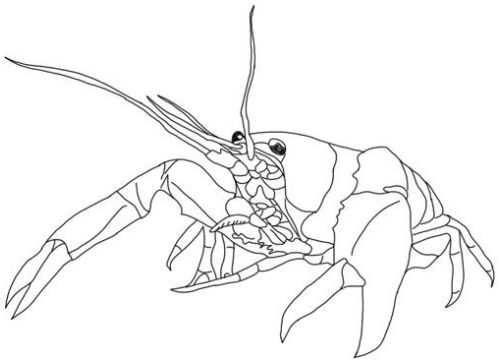
The findings of this PhD, have contributed important knowledge to invasion biology of crayfishes, particularly signal crayfish in upland stream ecosystems. This study on non-native signal crayfish in upland streams shows both generic and novel findings that justify the selection of signal crayfish as a model invasive species to understand different aspects of biological invasion. Apart from already-known impacts on indigenous biota (e.g. direct predation on biota and alteration of ecosystem processes) study on this model invasive species also reveals the density-dependent, medium to long-term impacts on multiple components of aquatic ecosystems and the varying roles of personality traits, population and habitat characteristics on invasive animal dispersal. This study also contributes evidence to the concept of ‘lag’ phases or time span after introduction and before impacts become evident in invasion ecology, in this case on different components of upland stream ecosystems. Thus, it is believed that this study on non-native signal crayfish as a model invasive species has contributed towards understanding the principles and theories of invasion ecology (Lockwood *et al.*, 2013; Enders *et al.*, 2018).

The evidence obtained in this thesis shows similarities and differences of signal crayfish responses in upland river systems to those in

lowland river systems. Signal crayfish have a relatively broad ecological niche and are tolerant of a wide range of environmental conditions, contributing to their success as invaders. The rate of their spread across Europe, and the strong ecological effects observed in this and other studies, suggest that they will have irreversible effects on many European freshwater ecosystems. They also have major economic impacts in some cases through, for example, destabilising river banks due to their burrowing behaviour. Long-term research at control and impacted sites needs to be continued, as suggested by Manchester and Bullock (2000), in order to document the extent of future changes caused by non-native crayfishes.

Although various control methods have been considered to stop or slow down invasive crayfish invasion it is evident that no single method can yield the desired solution to the problem (Freeman *et al.*, 2010). Therefore, further studies are needed in this regard, with a view to improving the performance of existing methods or inventing a new method that will be more effective. Dispersal and colonisation of invasive species is a key factor in understanding the replacement of inferior competitors and thus, study on potential factors that drive the invasion dynamics of a species may be important, especially for management plans for controlling invasive species. Region-specific legislative provisions and rising public awareness through formal and informal education can play a vital role in managing the non-natives. Obviously prevention of non-native species introduction is the most desirable but if the species is introduced it may be possible to prevent their establishment and subsequent spread through early detection (Juette *et al.*, 2014). Application of modern technique (e.g. eDNA) can effectively be used in detecting presence of non-native species including crayfish (Davison *et al.*, 2019; Robinson *et al.*, 2019). This technique may be used for early detection of invasive crayfish in order to ensure best possible management. For non-natives already established in habitats, regular monitoring and integrated control measures should be employed as no single “silver bullet” exists. The results of this study may be of help to enrich the regional database on non-native crayfish or invasive species as well as help improving management techniques by integrating crayfish

personality and other factors driving its invasion in its management, especially in upland stream ecosystems.



APPENDICES

Appendix I: Chapter Two supplementary tables

TABLE S2.1 Recorded bullhead densities in UK rivers.

River	Density (m^{-2})	Reference
Devil's Brook, Dorset	5.3	Mills and Mann (1983)
River Tarrant, Dorset	75	Mann (1971); Mills and Mann (1983)
Great Ouse	1.3–14.7	Guan and Wiles (1997)
Mill Stream	0.8	Prenda *et al.* (1997)
Bere Stream	2.2	Prenda *et al.* (1997)
All UK rivers	0.00002–11.1	Environment Agency (2016)

TABLE S2.2 Similarity Percentage Analysis (SIMPER), based on Bray-Curtis dissimilarity index on abundance data of macroinvertebrate families in first control group C_1 (without crayfish or benthic fish) over time (before vs. after).

Families	Average	Ratio	Average abundance		*p*-value	Cumulative contribution
			Before	After		
Heptageniidae	0.031	1.01	9.18	9.27	0.91	10.57
Chironomidae	0.031	1.18	2.35	2.75	0.618	21.12
Simulidae	0.025	1.27	1.99	2.43	0.823	29.92
Baetidae	0.023	0.98	2.95	2.97	0.783	38.03
Dixidae	0.023	0.97	1.41	1.41	0.89	45.83
Nemouridae	0.018	1.17	1.57	1.40	0.771	52.17
Ephemerellidae	0.018	1.25	2.00	2.20	0.848	58.27
Caenidae	0.015	1.07	1.38	1.29	0.813	63.60
Leptophebiidae	0.013	0.69	0.49	0.57	0.6	67.94
Rhyacophilidae	0.012	1.25	1.00	0.91	0.683	72.24
Perlodidae	0.011	1.30	1.28	1.26	0.907	76.10
Leuctridae	0.011	1.43	2.89	2.86	0.898	79.90
Culicidae	0.010	0.98	0.63	0.57	0.899	83.19
Tipulidae	0.009	0.68	0.49	0.40	0.796	86.29
Hydropsychidae	0.008	1.17	2.64	2.27	0.442	89.20
Elmidae	0.008	1.53	1.78	1.68	0.742	92.06
Gammaridae	0.007	1.24	3.20	3.65	0.28	94.48
Perlidae	0.007	0.69	0.28	0.35	0.709	96.82
Hydrophilidae	0.007	0.68	0.28	0.35	0.635	99.10
Polycentropodidae	0.003	0.49	0.00	0.20	0.377	100.00

Average, Average dissimilarly; Ratio, Average dissimilarly / SD; Cumulative contribution, contribution to overall dissimilarly between communities over time

TABLE S2.3 Similarity Percentage Analysis (SIMPER), based on Bray-Curtis dissimilarity index on abundance data of macroinvertebrate families in second control group C_2 (with benthic fish but no crayfish) over time (before vs. after).

Families	Average	Ratio	Average abundance		*p*-value	Cumulative contribution
			Before	After		
Chironomidae	0.035	1.12	2.65	2.93	0.828	11.56
Heptageniidae	0.032	1.15	9.35	8.73	0.842	22.17
Simulidae	0.026	1.20	2.39	2.38	1	30.72
Baetidae	0.025	0.99	2.91	2.99	0.973	38.89
Nemouridae	0.022	1.25	1.66	1.36	0.857	46.01
Dixidae	0.019	1.13	1.20	0.69	0.563	52.36
Ephemerellidae	0.018	1.41	1.99	1.41	0.598	58.33
Caenidae	0.016	1.26	1.28	1.00	0.731	63.65
Hydropsychidae	0.016	1.14	2.47	1.43	**0.033**	68.92
Elmidae	0.013	1.27	1.58	1.09	0.441	73.32
Leuctridae	0.012	1.34	2.97	2.74	0.737	77.32
Rhyacophilidae	0.012	1.11	0.83	0.00	0.096	81.14
Leptophebiidae	0.011	0.68	0.45	0.40	0.875	84.72
Perlodidae	0.010	1.24	1.13	1.08	0.976	88.16
Gammaridae	0.010	1.54	3.43	3.28	0.763	91.44
Tipulidae	0.010	0.68	0.49	0.40	0.511	94.60
Culicidae	0.008	0.99	0.48	0.40	0.599	97.20
Hydrophilidae	0.005	0.67	0.20	0.20	0.890	98.70
Perlidae	0.004	0.49	0.28	0.00	0.180	100

Average, Average dissimilarly; Ratio, Average dissimilarly / SD; Cumulative contribution, contribution to overall dissimilarly between communities over time

TABLE S2.4 Similarity Percentage Analysis (SIMPER), based on Bray-Curtis dissimilarity index on abundance data of macroinvertebrate families in first treatment group T_1 (low crayfish density treatment, with benthic fish and four crayfish) over time (before vs. after).

Families	Average	Ratio	Average abundance		*p*-value	Cumulative contribution
			Before	After		
Heptageniidae	0.064	1.52	9.19	6.27	0.132	15.27
Chironomidae	0.037	0.99	2.72	2.68	0.985	24.22
Simulidae	0.030	1.32	2.04	1.29	0.817	31.51
Gammaridae	0.030	2.60	3.49	1.68	**0.011**	38.7
Baetidae	0.029	1.26	2.81	1.95	0.555	45.67
Hydropsychidae	0.025	1.45	2.74	1.40	0.134	51.70
Ephemerellidae	0.025	1.45	1.95	0.97	0.372	57.64
Nemouridae	0.021	1.48	1.51	1.28	0.576	62.79
Elmidae	0.021	1.88	1.89	0.60	**0.011**	67.87
Caenidae	0.020	1.20	1.37	0.69	0.476	72.59
Dixidae	0.019	0.80	1.17	0.00	**0.018**	77.08
Leuctridae	0.018	2.17	3.50	2.40	**0.011**	81.43
Perlodidae	0.017	1.42	1.13	0.28	0.139	85.50
Leptophebiidae	0.015	0.82	0.69	0.35	0.679	89.03
Rhyacophilidae	0.013	1.12	0.88	0.20	0.136	92.25
Tipulidae	0.011	0.68	0.57	0.28	0.488	94.89
Culicidae	0.008	0.74	0.55	0.00	**0.018**	96.90
Perlidae	0.007	0.67	0.35	0.20	0.462	98.69
Hydrophilidae	0.005	0.49	0.35	0.00	**0.018**	100

Average, Average dissimilarly; Ratio, Average dissimilarly / SD; Cumulative contribution, contribution to overall dissimilarly between communities over time

TABLE S2.5 Similarity Percentage Analysis (SIMPER), based on Bray-Curtis dissimilarity index on abundance data of macroinvertebrate families in second treatment group T_2 (medium crayfish density treatment, with benthic fish and eight crayfish) over time (before vs. after).

Families	Average	Ratio	Average abundance		*p*-value	Cumulative contribution
			Before	After		
Heptageniidae	0.078	1.71	9.64	5.79	0.052	15.84
Gammaridae	0.044	3.15	3.41	0.97	**0.006**	24.72
Baetidae	0.036	1.60	2.98	1.66	0.415	32.01
Chironomidae	0.036	1.17	3.42	2.76	1	39.19
Simulidae	0.034	1.36	2.16	0.93	0.497	46.13
Leuctridae	0.033	1.67	2.93	1.21	**0.023**	52.85
Ephemerellidae	0.031	1.47	2.04	0.55	0.083	59.08
Hydropsychidae	0.030	1.72	2.63	0.97	0.073	65.23
Elmidae	0.027	1.79	1.62	0.20	**0.015**	70.7
Perlodidae	0.024	1.58	1.46	0.20	**0.015**	75.56
Nemouridae	0.023	1.72	1.51	1.05	0.221	80.24
Caenidae	0.022	1.23	1.37	0.28	0.072	84.71
Dixidae	0.020	0.79	1.17	0.00	**0.006**	88.72
Rhyacophilidae	0.015	1.09	0.88	0.00	**0.006**	91.71
Leptophebiidae	0.014	0.82	0.65	0.28	0.860	94.6
Tipulidae	0.009	0.49	0.53	0.00	**0.006**	96.33
Culicidae	0.008	0.93	0.40	0.40	1	98.03
Perlidae	0.005	0.49	0.28	0.00	**0.006**	99.06
Hydrophilidae	0.005	0.49	0.28	0.00	**0.006**	100

Average, Average dissimilarly; Ratio, Average dissimilarly / SD; Cumulative contribution, contribution to overall dissimilarly between communities over time

TABLE S2.6 Similarity Percentage Analysis (SIMPER), based on Bray-Curtis dissimilarity index on abundance data of macroinvertebrate families in third treatment group T_3 (high density treatment, with benthic fish and 12 crayfish) over time (before vs. after).

Families	Average	Ratio	Average abundance		*p*-value	Cumulative contribution
			Before	After		
Heptageniidae	0.101	1.68	9.31	4.56	0.069	15.49
Gammaridae	0.070	5.13	3.51	0.20	**0.006**	26.15
Hydropsychidae	0.061	7.03	2.96	0.00	**0.006**	35.57
Baetidae	0.048	1.55	2.98	1.11	0.507	42.96
Chironomidae	0.046	1.18	2.54	1.89	1	50.02
Leuctridae	0.042	2.02	2.79	1.32	0.383	56.49
Simulidae	0.039	1.16	2.04	0.28	0.092	62.45
Ephemerellidae	0.036	1.57	1.89	0.28	0.117	67.96
Elmidae	0.031	1.72	1.61	0.20	**0.022**	72.73
Nemouridae	0.031	1.13	1.58	0.00	**0.006**	77.48
Perlodidae	0.027	1.64	1.28	0.00	**0.006**	81.59
Caenidae	0.026	1.18	1.34	0.00	**0.006**	85.6
Dixidae	0.025	0.80	1.23	0.00	**0.006**	89.47
Rhyacophilidae	0.019	1.16	0.98	0.00	**0.006**	92.41
Leptophebiidae	0.016	0.65	0.69	0.00	**0.006**	94.89
Culicidae	0.011	0.73	0.55	0.00	**0.006**	96.55
Tipulidae	0.009	0.49	0.53	0.00	**0.006**	97.99
Perlidae	0.007	0.49	0.35	0.00	**0.006**	99.1
Hydrophilidae	0.006	0.49	0.28	0.00	**0.006**	100

Average, Average dissimilarly; Ratio, Average dissimilarly / SD; Cumulative contribution, contribution to overall dissimilarly between communities over time

TABLE S2.7 Similarity Percentage Analysis (SIMPER), based on Bray-Curtis dissimilarity index on abundance data of macroinvertebrate families between two control groups C_1 (without benthic fish or crayfish) and C_2 (benthic fish only) at the end of the study.

Families	Average	Ratio	Average abundance		*p*-value	Cumulative contribution
			C_1	C_2		
Chironomidae	0.035	1.11	2.75	2.93	0.976	11.51
Heptageniidae	0.032	1.02	9.27	8.73	0.966	21.91
Simulidae	0.025	1.21	2.43	2.38	0.868	30.17
Baetidae	0.024	0.96	2.97	2.99	0.804	38.05
Dixidae	0.022	1.13	1.41	0.69	0.485	45.12
Ephemerellidae	0.020	1.49	2.20	1.41	0.422	51.66
Nemouridae	0.019	1.25	1.40	1.36	0.996	57.89
Caenidae	0.016	1.19	1.29	1.00	0.613	62.93
Hydropsychidae	0.014	1.14	2.27	1.43	0.133	67.42
Leptophebiidae	0.013	0.69	0.57	0.40	0.490	71.57
Rhyacophilidae	0.012	1.17	0.91	0.00	0.077	75.58
Leuctridae	0.012	1.32	2.86	2.74	0.940	79.45
Elmidae	0.011	1.13	1.68	1.09	0.478	83.12
Perlodidae	0.011	1.28	1.26	1.08	0.811	86.72
Gammaridae	0.010	1.59	3.65	3.28	0.471	89.87
Culicidae	0.009	1.07	0.57	0.40	0.524	92.74
Tipulidae	0.008	0.67	0.40	0.40	0.945	95.44
Hydrophilidae	0.006	0.68	0.35	0.20	0.521	97.54
Perlidae	0.005	0.49	0.35	0.00	0.166	99.1
Polycentropodidae	0.003	0.49	0.20	0.00	0.153	100

Average, Average dissimilarly; Ratio, Average dissimilarly / SD; Cumulative contribution, contribution to overall dissimilarly between communities of two groups

TABLE S2.8 Similarity Percentage Analysis (SIMPER), based on Bray-Curtis dissimilarity index on abundance data of macroinvertebrate families between C_1 (without benthic fish or crayfish) and T_1 (low density treatment, benthic fishes and four crayfish) at the end of the study.

Families	Average	Ratio	Average abundance		*p*-value	Cumulative contribution
			C_1	T_1		
Heptageniidae	0.065	1.56	9.27	6.27	0.124	15.64
Chironomidae	0.040	1.09	2.75	2.68	0.972	25.15
Gammaridae	0.032	3.72	3.65	1.68	**0.014**	32.91
Baetidae	0.031	1.32	2.97	1.95	0.485	40.25
Simulidae	0.030	1.35	2.43	1.29	0.553	47.4
Ephemerellidae	0.027	1.57	2.20	0.97	0.210	53.98
Dixidae	0.023	0.79	1.41	0.00	**0.023**	59.43
Hydropsychidae	0.020	1.46	2.27	1.40	0.472	64.3
Nemouridae	0.020	1.46	1.40	1.28	0.745	69.03
Caenidae	0.019	1.20	1.29	0.69	0.410	73.52
Perlodidae	0.018	1.40	1.26	0.28	0.102	77.96
Elmidae	0.017	1.42	1.68	0.60	**0.022**	82.14
Leptophebiidae	0.015	0.68	0.57	0.35	0.503	85.66
Rhyacophilidae	0.014	1.24	0.91	0.20	0.071	88.98
Leuctridae	0.012	1.28	2.86	2.40	0.198	91.97
Culicidae	0.009	0.80	0.57	0.00	**0.023**	94.05
Tipulidae	0.009	0.69	0.40	0.28	0.991	96.12
Perlidae	0.007	0.67	0.35	0.20	0.441	97.89
Hydrophilidae	0.006	0.49	0.35	0.00	**0.023**	99.22
Polycentropodidae	0.003	0.49	0.20	0.00	**0.023**	100

Average, Average dissimilarly; Ratio, Average dissimilarly / SD; Cumulative contribution, contribution to overall dissimilarly between communities of two groups

TABLE S2.9 Similarity Percentage Analysis (SIMPER), based on Bray-Curtis dissimilarity index on abundance data of macroinvertebrate families between C_1 (without benthic fish or crayfish) and T_2 (medium crayfish density treatment, benthic fishes and eight crayfish) at the end of the study.

Families	Average	Ratio	Average abundance		*p*-value	Cumulative contribution
			C_1	T_2		
Heptageniidae	0.074	1.66	9.27	5.79	0.067	14.73
Gammaridae	0.049	3.46	3.65	0.97	**0.006**	24.4
Chironomidae	0.042	1.21	2.75	2.76	0.989	32.76
Baetidae	0.036	1.56	2.97	1.66	0.402	39.92
Ephemerellidae	0.034	1.59	2.20	0.55	0.058	46.68
Simulidae	0.033	1.47	2.43	0.93	0.254	53.2
Leuctridae	0.032	1.65	2.86	1.21	0.057	59.49
Elmidae	0.027	2.05	1.68	0.20	0.018	64.77
Hydropsychidae	0.025	1.58	2.27	0.97	0.283	69.82
Dixidae	0.025	0.79	1.41	0.00	**0.006**	74.73
Nemouridae	0.021	1.76	1.40	1.05	0.266	78.97
Caenidae	0.021	1.25	1.29	0.28	0.057	83.2
Perlodidae	0.020	1.49	1.26	0.20	**0.041**	87.25
Rhyacophilidae	0.016	1.17	0.91	0.00	**0.006**	90.32
Leptophebiidae	0.015	0.66	0.57	0.28	0.491	93.4
Culicidae	0.011	1.09	0.57	0.40	0.952	95.64
Tipulidae	0.006	0.49	0.40	0.00	**0.006**	96.9
Perlidae	0.006	0.49	0.35	0.00	**0.006**	98.1
Hydrophilidae	0.006	0.49	0.35	0.00	**0.006**	99.3
Polycentropodidae	0.004	0.49	0.20	0.00	**0.006**	100

Average, Average dissimilarly; Ratio, Average dissimilarly / SD; Cumulative contribution, contribution to overall dissimilarly between communities of two groups

TABLE S2.10 Similarity Percentage Analysis (SIMPER), based on Bray-Curtis dissimilarity index on abundance data of macroinvertebrate families between C_1 (without benthic fish or crayfish) and T_3 (high density treatment, benthic fishes and 12 crayfish) at the end of the study.

Families	Average	Ratio	Average abundance		*p*-value	Cumulative contribution
			C_1	T_3		
Heptageniidae	0.098	1.63	9.27	4.56	0.092	15.33
Gammaridae	0.072	5.41	3.65	0.20	**0.012**	26.56
Chironomidae	0.051	1.23	2.75	1.89	1	34.5
Baetidae	0.047	1.63	2.97	1.11	0.472	41.86
Hydropsychidae	0.046	6.80	2.27	0.00	**0.012**	49.08
Simulidae	0.045	1.59	2.43	0.28	**0.036**	56.04
Ephemerellidae	0.043	1.67	2.20	0.28	**0.046**	62.69
Leuctridae	0.033	1.47	2.86	1.32	0.799	67.87
Elmidae	0.031	2.06	1.68	0.20	0.053	72.67
Dixidae	0.028	0.80	1.41	0.00	**0.012**	77.1
Nemouridae	0.027	1.16	1.40	0.00	**0.012**	81.3
Perlodidae	0.026	1.65	1.26	0.00	**0.012**	85.36
Caenidae	0.025	1.19	1.29	0.00	**0.012**	89.28
Rhyacophilidae	0.018	1.17	0.91	0.00	**0.012**	92.04
Leptophebiidae	0.015	0.49	0.57	0.00	**0.012**	94.41
Culicidae	0.011	0.80	0.57	0.00	**0.012**	96.08
Tipulidae	0.007	0.49	0.40	0.00	**0.012**	97.20
Perlidae	0.007	0.49	0.35	0.00	**0.012**	98.29
Hydrophilidae	0.007	0.49	0.35	0.00	**0.012**	99.37
Polycentropodidae	0.004	0.49	0.20	0.00	**0.012**	100

Average, Average dissimilarly; Ratio, Average dissimilarly / SD; Cumulative contribution, contribution to overall dissimilarly between communities of two groups

TABLE S2.11 Similarity Percentage Analysis (SIMPER), based on Bray-Curtis dissimilarity index on abundance data of macroinvertebrate families between C_2 (benthic fish only) and T_1 (low density treatment, benthic fishes and four crayfish) at the end of the study.

Families	Average	Ratio	Average abundance		*p*-value	Cumulative contribution
			C_2	T_1		
Heptageniidae	0.065	1.60	8.73	6.27	0.126	16.97
Chironomidae	0.044	1.03	2.93	2.68	0.928	28.45
Simulidae	0.035	1.32	2.38	1.29	0.465	37.58
Baetidae	0.035	1.41	2.99	1.95	0.428	46.59
Gammaridae	0.030	1.97	3.28	1.68	**0.010**	54.31
Nemouridae	0.022	1.42	1.36	1.28	0.758	60.01
Ephemerellidae	0.020	1.34	1.41	0.97	0.963	65.28
Hydropsychidae	0.020	1.29	1.43	1.40	0.913	70.49
Perlodidae	0.018	1.49	1.08	0.28	0.116	75.21
Caenidae	0.018	1.19	1.00	0.69	0.757	79.83
Elmidae	0.014	1.20	1.09	0.60	0.367	83.36
Dixidae	0.013	0.79	0.69	0.00	**0.026**	86.77
Leptophebiidae	0.012	0.69	0.40	0.35	0.992	90.01
Leuctridae	0.011	1.22	2.74	2.40	0.367	93.01
Tipulidae	0.009	0.69	0.40	0.28	0.986	95.46
Culicidae	0.007	0.79	0.40	0.00	**0.042**	97.22
Rhyacophilidae	0.004	0.49	0.00	0.20	0.968	98.21
Hydrophilidae	0.004	0.49	0.20	0.00	**0.042**	99.14
Perlidae	0.003	0.49	0.00	0.20	0.990	100

Average, Average dissimilarly; Ratio, Average dissimilarly / SD; Cumulative contribution, contribution to overall dissimilarly between communities over time

TABLE S2.12 Similarity Percentage Analysis (SIMPER), based on Bray-Curtis dissimilarity index on abundance data of macroinvertebrate families between C_2 (benthic fish only) and T_2 (medium density treatment, benthic fishes and eight crayfish) at the end of the study.

Families	Average	Ratio	Average abundance		*p*-value	Cumulative contribution
			C_2	T_2		
Heptageniidae	0.075	1.78	8.73	5.79	0.070	16.39
Chironomidae	0.048	1.19	2.93	2.76	1	26.93
Gammaridae	0.048	2.31	3.28	0.97	**0.007**	37.37
Baetidae	0.041	1.65	2.99	1.66	0.221	46.3
Simulidae	0.038	1.31	2.38	0.93	0.223	54.62
Leuctridae	0.033	1.69	2.74	1.21	**0.040**	61.81
Ephemerellidae	0.024	1.32	1.41	0.55	0.266	67.08
Nemouridae	0.023	1.51	1.36	1.05	0.334	72.09
Hydropsychidae	0.022	1.38	1.43	0.97	0.953	76.84
Elmidae	0.020	1.39	1.09	0.20	0.064	81.16
Perlodidae	0.020	1.46	1.08	0.20	0.055	85.43
Caenidae	0.019	1.18	1.00	0.28	0.221	89.57
Rhyacophilidae	0.014	0.79	0.69	0.00	**0.007**	92.73
Leptophebiidae	0.013	0.69	0.40	0.28	1	95.55
Culicidae	0.010	0.93	0.40	0.40	1	97.65
Tipulidae	0.007	0.49	0.40	0.00	**0.007**	99.14
Hydrophilidae	0.004	0.49	0.20	0.00	**0.007**	100

Average, Average dissimilarly; Ratio, Average dissimilarly / SD; Cumulative contribution, contribution to overall dissimilarly between communities over time

TABLE S2.13 Similarity Percentage Analysis (SIMPER), based on Bray-Curtis dissimilarity index on abundance data of macroinvertebrate families between C_2 (benthic fish only) and T_3 (high density treatment, benthic fishes and 12 crayfish) at the end of the study.

Families	Average	Ratio	Average abundance		*p*-value	Cumulative contribution
			C_2	T_3		
Heptageniidae	0.101	1.65	8.73	4.56	0.085	17.14
Gammaridae	0.074	3.35	3.28	0.20	**0.006**	29.7
Chironomidae	0.061	1.34	2.93	1.89	1	39.96
Baetidae	0.055	1.70	2.99	1.11	0.346	49.21
Simulidae	0.052	1.31	2.38	0.28	**0.037**	57.98
Leuctridae	0.035	1.46	2.74	1.32	0.740	63.82
Hydropsychidae	0.033	1.80	1.43	0.00	**0.006**	69.35
Ephemerellidae	0.031	1.39	1.41	0.28	0.172	74.61
Nemouridae	0.029	1.11	1.36	0.00	**0.006**	79.51
Perlodidae	0.026	1.71	1.08	0.00	**0.006**	83.89
Elmidae	0.023	1.37	1.09	0.20	0.120	87.83
Caenidae	0.022	1.13	1.00	0.00	**0.006**	91.60
Rhyacophilidae	0.017	0.79	0.69	0.00	**0.006**	94.49
Leptophebiidae	0.012	0.49	0.40	0.00	**0.006**	96.46
Culicidae	0.009	0.79	0.40	0.00	**0.006**	97.9
Tipulidae	0.008	0.49	0.40	0.00	**0.006**	99.22
Hydrophilidae	0.005	0.49	0.20	0.00	**0.006**	100

Average, Average dissimilarly; Ratio, Average dissimilarly / SD; Cumulative contribution, contribution to overall dissimilarly between communities of two groups

Appendix II: Chapter Three supplementary tables

TABLE S3.1 Results of the trial experiments, obtained through Linear Mixed-Effects Modelling (LMM; crayfish IDs were considered random effect in model).

Behaviours	**Comparisons**	
	Field vs. Lab (Week1) (*N*=15)	**Lab, Week1 vs. Week3 (*N*=33)**
Activity	*F*=51.93; *P*<0.001	*F*=20.19; *P*<0.001
Distance moved	*F*=28.41; *P*<0.001	*F*=38.99; *P*<0.001
Area (%) explored	*F*=10.71; *P*=0.006	*F*=12.76, *P*=0.003

Appendix III: Chapter Four supplementary tables & figures

Table S4.1 Canopy cover scale used for measuring canopy covers of the streams surveyed in 2011 and 2018; modified from Ream (2010).

Canopy cover category	Description
0	Vegetation height <1 m on both banks
1	Vegetation height <2 m on both banks
2	Vegetation height >2 m on one bank only
3	Vegetation height >2 m on both banks
4	Vegetation height >2 m on both banks and overhanging branches
5	Dense overhead cover

Table S4.2 Similarity Percentage Analysis (SIMPER), based on Bray-Curtis dissimilarity index on presence-absence data of macroinvertebrate families in pre-invaded streams (S_1, 2011 and 2018, showing change in proportion of samples in which family is present).

Family	Average dissimilarity	Average occurrence		*p*-value	Cumulative % contribution to dissimilarity
		2011	2018		
Rhyacophilidae[1]	0.0268	0.13	0.75	**0.032**	7.24
Astacidae	0.0205	0.50	1.00	**0.042**	12.79
Tipuloidea	0.0204	1.00	0.50	**0.038**	18.31
Polycentropidae	0.0202	0.50	0.63	0.554	23.77
Leptophlebiidae	0.0202	0.38	0.50	0.655	29.23
Perlodidae	0.0197	0.00	0.50	0.052	34.54
Elmidae	0.0185	0.75	0.63	0.472	39.53
Ephemerellidae	0.0167	0.38	0.25	0.921	44.04
Nemouridae	0.0154	0.75	0.75	0.742	48.20
Oligochaeta	0.0151	0.25	0.25	0.720	52.28
Hydropsychidae	0.0142	0.00	0.38	0.148	56.13
Sphaeriidae	0.0141	0.38	0.00	0.187	59.95
Leuctridae	0.0131	0.88	0.75	0.356	63.48
Ephemeridae	0.0123	0.13	0.25	0.282	66.82
Valvatidae	0.0117	0.25	0.13	0.854	69.99
Philopotamidae	0.0107	0.00	0.25	0.183	72.89
Asellidae	0.0090	0.25	0.00	0.369	75.32
Dytiscidae	0.0090	0.25	0.00	0.369	77.76
Gammaridae	0.0089	0.88	0.88	0.663	80.16
Limnephilidae	0.0089	0.13	0.13	0.663	82.57
Perlidae	0.0082	0.13	0.13	0.762	84.78
Odontoceridae	0.0082	0.13	0.13	0.762	87.00
Baetidae	0.0056	1.00	0.88	0.389	88.52
Goeridae	0.0053	0.13	0.00	0.475	89.96
Simuliidae	0.0049	1.00	0.88	0.471	91.29
Ancylidae	0.0049	0.13	0.00	0.547	92.62
Hydrobiidae	0.0049	0.13	0.00	0.547	93.95
Heptageniidae	0.0049	0.88	1.00	0.547	95.27
Caenidae	0.0047	0.13	0.00	0.594	96.55
Planorbidae	0.0046	0.00	0.13	0.574	97.78
Lymnaeidae	0.0041	0.13	0.00	0.752	98.89
Gyrinidae	0.0041	0.13	0.00	0.752	100.00

[1], including Glossosomatidae as 'composite taxa' (Armitage *et al.*, 1983)

Table S4.3 Similarity percentage analysis (SIMPER), based on Bray-Curtis dissimilarity index on presence-absence data of macroinvertebrate families in newly-invaded streams (S_1, 2011 and 2018 showing change in proportion of samples in which family is present).

Family	Average dissimilarity	Average occurrence		*p*-value	Cumulative % contribution to dissimilarity
		2011	2018		
Lymnaeidae	0.0346	1.00	0.00	0.1	7.80
Limnephilidae	0.0277	0.33	0.67	1	14.06
Ancylidae	0.0233	0.67	0.00	0.1	19.33
Astacidae	0.0224	0.00	0.67	0.4	24.37
Dytiscidae	0.0193	0.67	0.33	0.5	28.74
Oligochaeta	0.0190	0.33	0.67	0.8	33.04
Hydrobiidae	0.0156	0.33	0.33	1	36.56
Ephemeridae	0.0156	0.33	0.33	1	40.09
Ephemerellidae	0.0156	0.67	0.67	1	43.62
Leuctridae	0.0156	0.67	0.67	1	47.15
Sphaeriidae	0.0154	0.33	0.33	1	50.61
Caenidae	0.0154	0.33	0.33	1	54.08
Nemouridae	0.0154	0.67	0.67	1	57.55
Polycentropidae	0.0154	0.33	0.33	1	61.02
Hydropsychidae	0.0152	0.33	0.33	1	64.46
Haliplidae	0.0151	0.33	0.33	1	67.86
Tipuloidea	0.0151	0.33	0.33	1	71.27
Perlodidae	0.0122	0.00	0.33	1	74.02
Glossiphoniidae	0.0117	0.33	0.00	0.2	76.65
Heptageniidae	0.0117	0.67	1.00	0.2	79.28
Perlidae	0.0117	0.33	0.00	0.2	81.91
Sialidae	0.0117	0.33	0.00	0.2	84.55
Sericostomatidae	0.0117	0.33	0.00	0.2	87.18
Phyrganeidae	0.0117	0.33	0.00	0.2	89.81
Simuliidae	0.0114	0.00	0.33	1	92.37
Neritidae	0.0113	0.33	0.00	0.3	94.92
Hydrophilidae	0.0113	0.33	0.00	0.3	97.46
Rhyacophilidae[1]	0.0113	0.67	1.00	0.3	100.00

[1], including Glossosomatidae as 'composite taxa' (Armitage *et al.*, 1983)

Table S4.4 Similarity Percentage Analysis (SIMPER), based on Bray-Curtis dissimilarity index on presence-absence data of macroinvertebrate families in uninvaded streams (S_1, 2011 and 2018 showing change in proportion of samples in which family is present).

Family	Average dissimilarity	Average occurrence		*p*-value	Cumulative % contribution to dissimilarity
		2011	2018		
Polycentropidae	0.0217	0.14	0.71	**0.024**	5.99
Rhyacophilidae[1]	0.0213	0.29	0.86	0.092	11.88
Valvatidae	0.0189	0.57	0.14	0.053	17.11
Ephemeridae	0.0175	0.43	0.71	0.454	21.95
Dytiscidae	0.0174	0.71	0.43	0.499	26.75
Tipuloidea	0.0161	0.57	0.57	0.945	31.19
Perlidae	0.0154	0.14	0.43	0.227	35.43
Odontoceridae	0.0153	0.29	0.43	0.554	39.65
Oligochaeta	0.0150	0.14	0.43	**0.050**	43.80
Sphaeriidae	0.0149	0.29	0.43	0.956	47.90
Limnephilidae	0.0142	0.43	0.00	0.167	51.81
Hydropsychidae	0.0141	0.14	0.43	0.27	55.70
Planorbidae	0.0138	0.29	0.25	0.632	59.51
Lymnaeidae	0.0130	0.29	0.29	0.944	63.09
Nemouridae	0.0121	0.71	0.86	0.934	66.43
Simuliidae	0.0118	0.71	0.86	0.939	69.69
Heptageniidae	0.0110	0.71	0.86	0.872	72.72
Hydrobiidae	0.0104	0.14	0.29	0.635	75.59
Glossiphoniidae	0.0104	0.14	0.29	0.635	78.46
Elmidae	0.0103	0.71	1.00	0.367	81.29
Erpobdeliidae	0.0095	0.00	0.29	**0.036**	83.91
Leptophlebiidae	0.0085	0.86	0.86	0.512	86.26
Philopotamidae	0.0080	0.14	0.14	0.864	88.45
Ancylidae	0.0073	0.14	0.14	0.94	90.48
Ephemerellidae	0.0069	1.00	0.92	**0.045**	92.37
Leuctridae	0.0055	1.00	0.86	**0.029**	93.89
Gammaridae	0.0047	0.86	1.00	0.905	95.19
Sericostomatidae	0.0047	0.14	0.00	0.905	96.49
Sialidae	0.0044	0.14	0.00	0.94	97.75
Perlodidae	0.0040	0.00	0.14	0.137	100.00

[1], including Glossosomatidae as 'composite taxa' (Armitage *et al.*, 1983)

Table S4.5 Similarity Percentage Analysis (SIMPER), based on Bray-Curtis dissimilarity index of abundance of macroinvertebrate families in signal crayfish–invaded and uninvaded sections of Alwent Beck and Thorsgill Beck in 2018.

Family	Average dissimilarity	Average abundance		*p*-value	Cumulative % contribution to dissimilarity
		Uninvaded	Invaded		
Elmidae	0.0583	5.04	2.17	**0.001**	12.96
Hydrobiidae	0.0450	2.42	0.00	**0.001**	22.95
Gammaridae	0.0316	3.04	2.26	0.688	29.97
Chironomidae	0.0236	1.20	0.97	0.604	35.21
Ephemerellidae	0.0223	1.44	0.63	**0.034**	40.18
Heptageniidae	0.0213	1.52	1.73	1	44.90
Nemouridae	0.0212	1.85	1.80	1	49.61
Rhyacophilidae	0.0202	1.25	0.91	0.677	54.11
Leuctridae	0.0180	2.64	2.31	0.93	58.11
Hydropsychidae	0.0177	0.87	0.82	0.999	62.04
Baetidae	0.0167	1.93	1.95	0.657	65.75
Emphididae	0.0151	0.63	0.74	1	69.12
Odontoceridae	0.0149	0.77	0.00	**0.001**	72.44
Sphaeriidae	0.0129	0.65	0.00	**0.001**	75.30
Tipuloidea	0.0123	0.70	0.91	0.976	78.04
Polycentropidae	0.0119	0.43	0.37	1	80.69
Veliidae	0.0115	0.52	0.33	0.542	83.24
Psychodidae	0.0115	0.62	0.00	**0.001**	85.80
Dytiscidae	0.0105	0.48	0.33	0.734	88.13
Oligochaeta	0.0089	0.37	0.25	0.495	90.11
Ephemeridae	0.0088	0.33	0.25	0.987	92.08
Simuliidae	0.0083	0.28	0.20	0.979	93.93
Ancylidae	0.0076	0.39	0.00	**0.001**	95.61
Halipidae	0.0072	0.25	0.25	1	97.21
Valvatidae	0.0072	0.33	0.00	**0.001**	98.81
Caenidae	0.0053	0.25	0.00	**0.001**	100.00

Table S4.6 Similarity Percentage Analysis (SIMPER), based on Bray-Curtis dissimilarity index on ordinal abundance data of macroinvertebrate families before and after signal crayfish invasion in three invaded streams (Deepdale Beck, River Balder and River Lune; Environment Agency data, 1990–2017).

Family	Average dissimilarity	Average abundance		*p*-value	Cumulative % contribution to dissimilarity
		Before	After		
Lepidostomatidae	0.0128	0.86	0.39	**0.001**	3.34
Caenidae	0.0123	0.85	0.45	**0.003**	6.54
Ancylidae	0.0122	0.77	0.33	**0.001**	9.71
Perlidae	0.0119	0.69	0.25	**0.001**	12.81
Chloroperlidae	0.0110	0.59	0.63	0.487	15.67
Sericostomatidae	0.0106	0.68	0.58	0.134	18.45
Polycentropodidae	0.0104	0.86	0.52	**0.007**	21.17
Limnephilidae	0.0104	0.73	0.55	0.072	23.88
Gammaridae	0.0104	1.06	1.08	0.504	26.59
Leptophlebiidae	0.0103	1.00	0.73	**0.003**	29.28
Tipuloidea	0.0102	0.92	0.83	0.337	31.93
Perlodidae	0.0099	0.84	0.93	0.162	34.51
Hydroptilidae	0.0098	0.40	0.43	0.426	37.08
Sphaeriidae	0.0096	0.56	0.34	**0.050**	39.58
Empididae	0.0094	0.53	0.34	0.190	42.05
Hydracarina	0.0093	0.45	0.41	0.552	44.48
Hydrophilidae	0.0088	0.25	0.48	**0.019**	46.76
Gyrinidae	0.0086	0.47	0.17	**0.015**	49.00
Leptoceridae	0.0086	0.45	0.26	0.225	51.24
Simuliidae	0.0085	0.99	1.00	0.356	53.45
Oligochaeta	0.0081	1.36	1.10	**0.001**	55.55
Hydrobiidae	0.0080	0.44	0.08	**0.017**	57.63
Odontoceridae	0.0077	0.31	0.34	0.381	59.64
Dytiscidae	0.0076	0.38	0.18	0.111	61.61
Ephemeridae	0.0075	0.36	0.24	0.307	63.57
Rhyacophilidae	0.0074	1.18	0.97	**0.020**	65.49
Psychomyiidae	0.0074	0.31	0.30	0.488	67.41
Nemouridae	0.0071	1.21	1.04	0.070	69.27
Ceratopogonidae	0.0071	0.36	0.21	0.286	71.11
Ephemerellidae	0.0070	0.10	0.38	**0.004**	72.94
Scirtidae	0.0064	0.24	0.24	0.517	74.62
Glossosomatidae	0.0063	0.09	0.37	**0.015**	76.27
Lymnaeidae	0.0062	0.27	0.16	0.439	77.88
Rhagionidae	0.0061	0.25	0.21	0.484	79.47
Heptageniidae	0.0061	1.42	1.55	**0.007**	81.05

Family	Average dissimilarity	Average abundance		*p*-value	Cumulative % contribution to dissimilarity
		Before	After		
Leuctridae	0.0060	1.19	1.24	0.296	82.62
Taeniopterygidae	0.0058	0.13	0.23	0.147	84.13
Goeridae	0.0054	0.08	0.29	**0.016**	85.53
Brachycentridae	0.0051	0.27	0.05	0.150	86.86
Elmidae	0.0050	1.33	1.27	0.233	88.17
Chironomidae	0.0048	1.37	1.42	0.191	89.41
Baetidae	0.0044	1.42	1.57	**0.004**	90.55
Hydropsychidae	0.0042	1.38	1.27	0.062	91.63
Glossiphoniidae	0.0041	0.16	0.11	0.482	92.70
Asellidae	0.0037	0.16	0.05	0.601	93.67
Psychodidae	0.0034	0.12	0.11	0.468	94.54
Planariidae	0.0031	0.14	0.05	0.541	95.35
Hydraenidae	0.0029	0.14	0.03	0.553	96.11
Nematoda	0.0025	0.04	0.13	0.110	96.76
Astacidae	0.0021	0.06	0.05	0.436	97.65
Sialidae	0.0018	0.08	0.03	0.732	98.31
Gordiidae	0.0012	0.04	0.03	0.419	98.62
Erpobdellidae	0.0010	0.02	0.03	0.450	98.89
Muscidae	0.0010	0.04	0.03	0.401	99.16
Philopotamidae	0.0009	0.02	0.03	0.428	99.39
Crangonyctidae	0.0005	0.00	0.03	0.410	99.53
Culicidae	0.0005	0.00	0.03	0.418	99.66
Beraeidae	0.0004	0.00	0.03	0.415	99.76
Thaumaleidae	0.0003	0.02	0.00	0.448	99.84
Capniidae	0.0003	0.02	0.00	0.442	99.92
Planorbidae	0.0003	0.02	0.00	0.442	100.00

Table S4.7 Similarity Percentage Analysis (SIMPER), based on Bray-Curtis dissimilarity index on ordinal abundance data of macroinvertebrate families, before and after mid-1997 (in respect to invasion period in nearby streams) in three uninvaded streams (Aldbrough Beck, Clow Beck and River Greta; Environment Agency data, 1990–2017).

Family	Average dissimilarity	Average abundance		*p*-value	Cumulative % contribution to dissimilarity
		Before	After		
Glossosomatidae	0.0156	0.12	1.06	**0.001**	3.65
Ephemeridae	0.0116	0.62	0.95	**0.026**	6.37
Hydroptilidae	0.0114	0.57	1.03	**0.003**	9.04
Hydrobiidae	0.0111	0.86	0.88	0.886	11.63
Lepidostomatidae	0.0109	0.61	0.97	**0.018**	14.18
Hydracarina	0.0107	0.43	0.91	**0.002**	16.67
Leptophlebiidae	0.0107	0.52	0.79	**0.002**	19.16
Simuliidae	0.0105	0.76	1.01	0.063	21.63
Chironomidae	0.0105	1.04	1.00	0.843	24.08
Perlodidae	0.0105	0.60	0.41	0.216	26.52
Nemouridae	0.0104	0.62	0.44	0.235	28.96
Leuctridae	0.0104	0.73	0.88	0.172	31.39
Ancylidae	0.0103	0.66	0.87	0.097	33.80
Empididae	0.0103	0.33	0.74	0.003	36.20
Sphaeriidae	0.0102	0.55	0.70	0.514	38.59
Caenidae	0.0101	0.61	0.63	0.570	40.96
Limnephilidae	0.0101	0.65	0.80	0.330	43.32
Dytiscidae	0.0101	0.72	0.35	**0.001**	45.67
Lymnaeidae	0.0100	0.49	0.66	0.114	48.02
Glossiphoniidae	0.0100	0.68	0.79	0.304	50.37
Erpobdellidae	0.0100	0.58	0.57	0.691	52.70
Polycentropodidae	0.0095	0.59	0.32	0.035	54.91
Ceratopogonidae	0.0095	0.56	0.57	0.590	57.12
Leptoceridae	0.0087	0.48	0.49	0.837	59.16
Asellidae	0.0086	0.36	0.46	0.262	61.17
Sericostomatidae	0.0086	0.93	0.91	0.690	63.17
Gyrinidae	0.0085	0.52	0.58	0.659	65.15
Perlidae	0.0084	0.52	0.31	0.085	67.13
Gammaridae	0.0084	1.16	1.33	0.162	69.09
Chloroperlidae	0.0080	0.40	0.28	0.316	70.95
Heptageniidae	0.0078	1.23	1.22	0.415	72.79
Rhyacophilidae	0.0070	0.99	0.83	0.209	74.41
Hydraenidae	0.0068	0.08	0.39	**0.006**	76.01
Planorbidae	0.0068	0.06	0.46	**0.001**	77.60
Muscidae	0.0067	0.30	0.30	0.841	79.17

Family	Average dissimilarity	Average abundance		*p*-value	Cumulative % contribution to dissimilarity
		Before	After		
Oligochaeta	0.0066	1.37	1.46	0.234	80.73
Goeridae	0.0063	0.08	0.41	**0.006**	82.19
Psychomyiidae	0.0058	0.11	0.37	**0.034**	83.55
Psychodidae	0.0058	0.12	0.34	**0.032**	84.91
Tipuloidea	0.0057	1.16	1.14	0.431	86.23
Planariidae	0.0055	0.21	0.26	0.526	87.53
Hydrophilidae	0.0048	0.27	0.05	**0.013**	88.66
Haliplidae	0.0046	0.28	0.05	**0.021**	89.73
Hydropsychidae	0.0042	1.33	1.30	0.244	90.72
Baetidae	0.0041	1.40	1.57	**0.001**	91.67
Odontoceridae	0.0038	0.14	0.14	0.689	92.56
Rhagionidae	0.0037	0.19	0.10	0.272	93.43
Sialidae	0.0037	0.14	0.16	0.766	94.29
Elmidae	0.0030	1.39	1.49	0.026	95.14
Scirtidae	0.0030	0.03	0.16	0.109	95.99
Taeniopterygidae	0.0025	0.11	0.08	**0.543**	96.84
Philopotamidae	0.0025	0.12	0.05	**0.284**	97.69
Nematoda	0.0025	0.06	0.13	0.387	98.28
Siphlonuridae	0.0011	0.07	0.00	0.215	98.55
Corixidae	0.0011	0.03	0.05	0.562	98.80
Dendrocoelidae	0.0008	0.06	0.00	0.223	98.99
Dugesiidae	0.0008	0.00	0.05	0.245	99.18
Valvatidae	0.0008	0.00	0.05	0.245	99.36
Brachycentridae	0.0007	0.00	0.05	0.303	99.53
Physidae	0.0005	0.03	0.00	0.434	99.65
Mesoveliidae	0.0005	0.00	0.03	0.512	99.76
Capniidae	0.0004	0.03	0.00	0.478	99.84
Stratiomyidae	0.0004	0.03	0.00	0.471	99.93
Helophoridae	0.0003	0.00	0.03	0.526	100.00

Table S4.8 Linear model summaries of changes in water quality parameters in different streams over time. Data points and trend lines for significant relationships are drawn in Figures S4.2–S4.10.

Parameter	Group	Stream	Linear regression model statistics		
			R^2	*F*	*P*
BOD	Invaded	Alwent Beck	0.06	11.01	**0.001**
		Deepdale Beck	0.02	1.71	0.196
		River Balder	0.04	7.78	**0.006**
		River Lune	0.04	8.07	**0.005**
	Uninvaded	Clow Beck	0.16	37.72	**<0.001**
		River Greta	0.002	0.14	0.708
DO	Invaded	Alwent Beck	0.01	1.05	0.307
		Deepdale Beck	0.002	0.15	0.701
		River Balder	0.004	0.48	0.494
		River Lune	0.0004	0.05	0.829
	Uninvaded	Clow Beck	0.03	3.57	0.061
		River Greta	0.003	0.64	0.423
Hardness	Invaded	Alwent Beck	0.07	4.63	**0.035**
		Deepdale Beck	0.02	0.74	0.395
		River Balder	0.01	0.79	0.377
		River Lune	0.12	13.41	**<0.001**
	Uninvaded	Clow Beck	0.04	2.79	0.100
		River Greta	0.04	3.26	0.075
Nitrogen	Invaded	Alwent Beck	0.19	35.31	**<0.001**
		Deepdale Beck	0.07	6.94	**0.010**
		River Balder	0.25	46.31	**<0.001**
		River Lune	0.20	34.05	**<0.001**
	Uninvaded	Clow Beck	0.40	92.77	**<0.001**
		River Greta	0.06	4.09	**0.048**
Ammonia	Invaded	Alwent Beck	0.01	2.41	0.123
		Deepdale Beck	0.02	2.15	0.146
		River Balder	0.07	12.56	**0.001**
		River Lune	0.06	8.75	**0.004**
	Uninvaded	Clow Beck	0.03	4.82	**0.030**
		River Greta	0.07	6.03	**0.016**
pH	Invaded	Alwent Beck	0.06	11.13	**0.001**
		Deepdale Beck	0.003	0.30	0.586
		River Balder	0.09	18.18	**<0.001**
		River Lune	0.02	2.31	0.130
	Uninvaded	Clow Beck	0.07	12.58	**0.001**
		River Greta	0.01	1.29	0.257
Temperature	Invaded	Alwent Beck	0.01	1.18	0.278
		Deepdale Beck	0.02	1.50	0.224
		River Balder	0.003	0.57	0.453
		River Lune	0.01	0.78	0.380
	Uninvaded	Clow Beck	0.002	0.38	0.540
		River Greta	0.001	0.18	0.668
Turbidity	Invaded	River Balder	0.04	3.85	**0.053**
		River Lune	0.06	6.98	**0.009**
Zinc	Invaded	Alwent Beck	0.02	2.57	0.112
		Deepdale Beck	0.001	0.07	0.788
		River Balder	0.04	6.99	**0.009**
		River Lune	0.04	5.99	**0.016**
	Uninvaded	Clow Beck	0.01	2.02	0.157
		River Greta	0.06	4.32	**0.041**

FIGURE S4.1A Study streams.

FIGURE S4.1B Study streams.

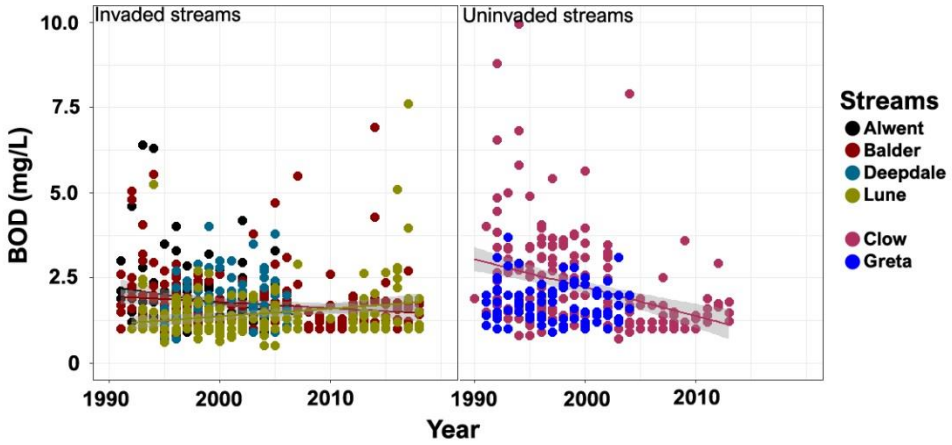


FIGURE S4.2 Change in biochemical oxygen demand (BOD, mg L^{-1}) over time in streams of the River Tees; linear fit with 95% confidence interval represented by grey-shaded areas for streams showing significant trends (Alwent Beck, River Balder, River Lune, River Greta).

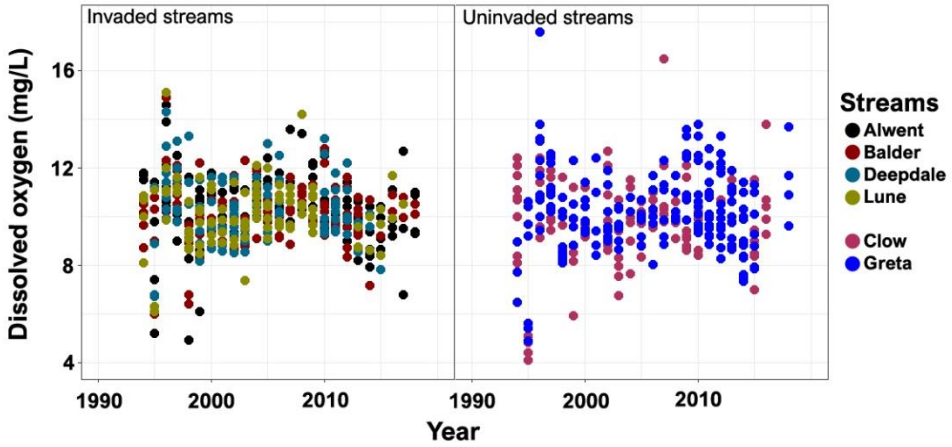


FIGURE S4.3 Change in dissolved oxygen (DO, mg L^{-1}) over time in streams of the River Tees.

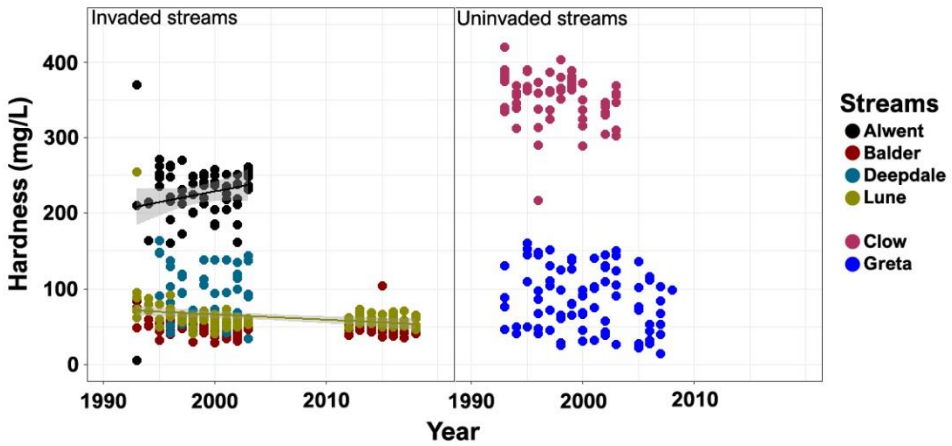


FIGURE S4.4 Change in total hardness (mg L^{-1}) over time in streams of the River Tees; linear fit with 95% confidence interval represented by grey-shaded areas for streams showing significant trends (Alwent Beck, River Lune).

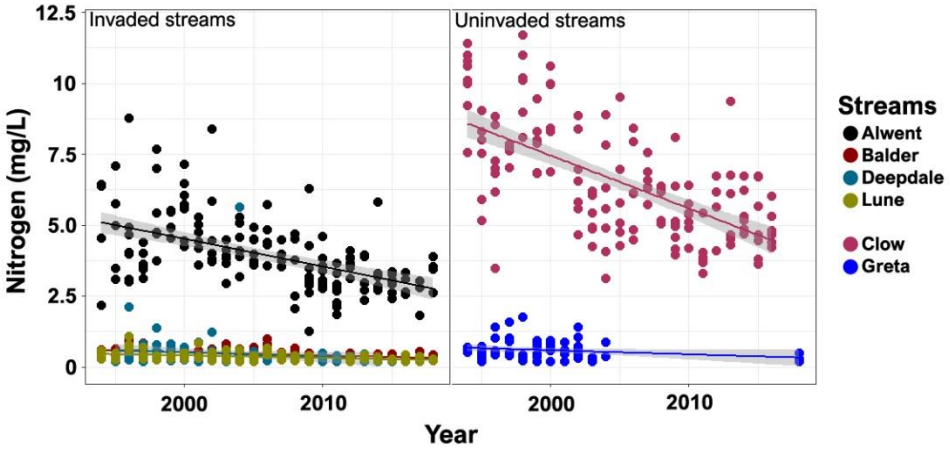


FIGURE S4.5 Change in total nitrogen (mg L^{-1}) over time in streams of the River Tees; linear fit with 95% confidence interval represented by grey-shaded areas for streams showing significant trends (all streams).

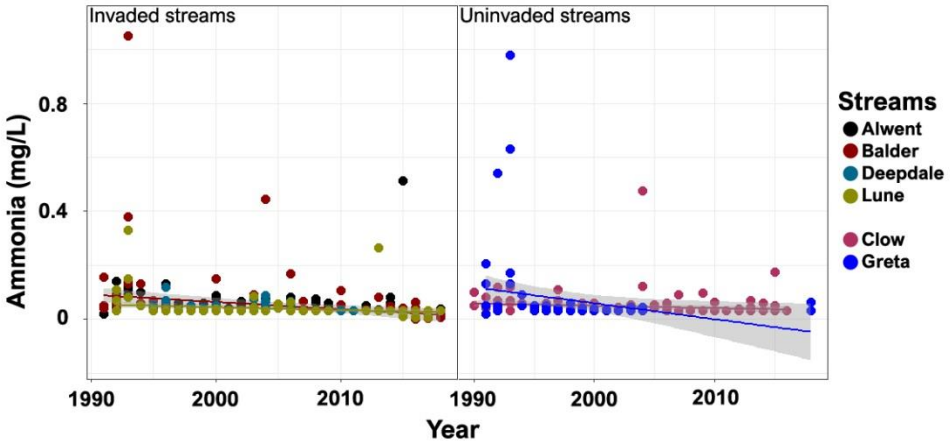


FIGURE S4.6 Change in ammonia (mg L^{-1}) over time in streams of the River Tees; linear fit with 95% confidence interval represented by grey-shaded areas for streams showing significant trends (River Balder, River Lune, Clow Beck, River Greta)

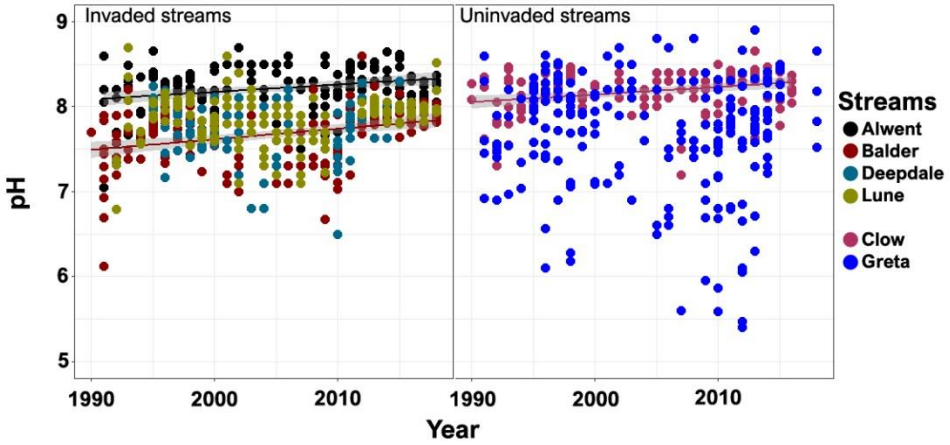


FIGURE S4.7 Change in pH over time in streams of the River Tees; linear fit with 95% confidence interval represented by grey-shaded areas for streams showing significant trends (Alwent Beck, River Balder, Clow Beck).

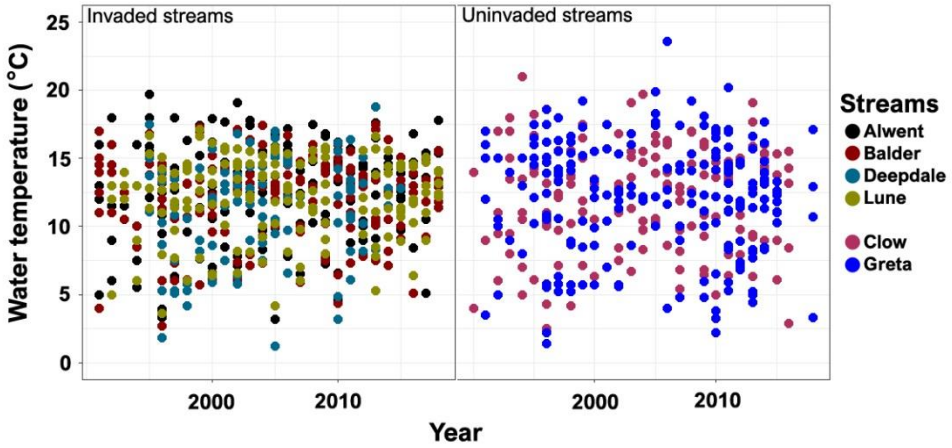


FIGURE S4.8 Change in water temperature (˚C) over time in different streams of the River Tees.

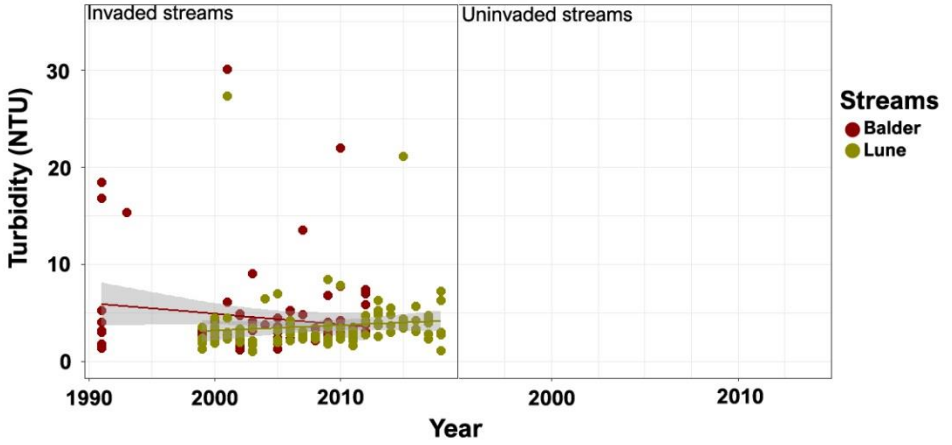


FIGURE S4.9 Change in water turbidity (NTU) over time in streams of the River Tees; linear fit with 95% confidence interval represented by grey-shaded areas for streams showing significant trends (River Balder and River Lune).

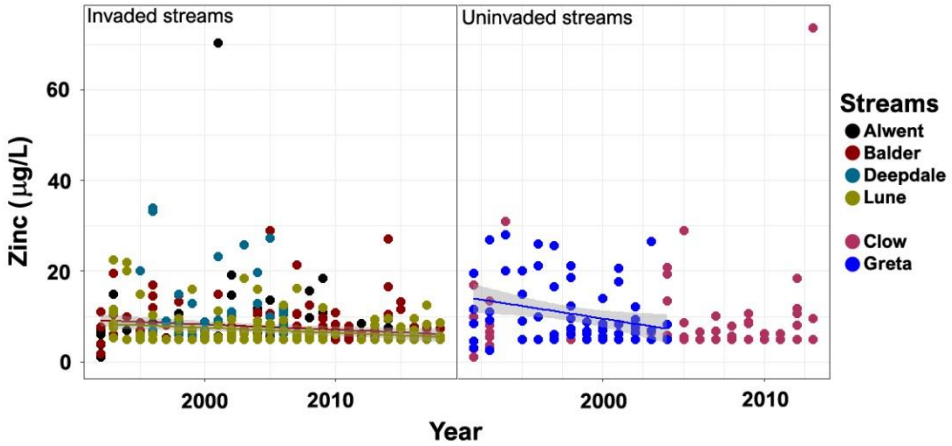
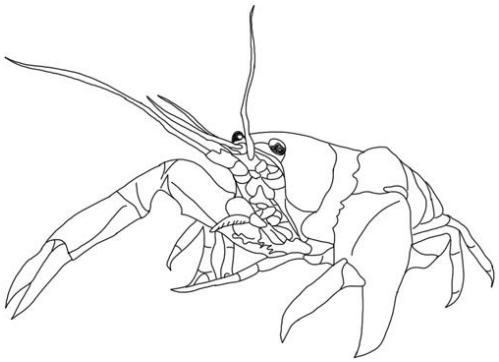


FIGURE S4.10 Change in zinc (µg L^{-1}) over time in streams of the River Tees; linear fit with 95% confidence interval represented by grey-shaded areas for streams showing significant trends (River Balder, River Lune, River Greta).



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